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12 JAN 2000 — **0000663.5**

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Kent
CT13 9NJ

6892673001

X

Patents ADP number *(if you know it)*

If the applicant is a corporate body, give the country/state of its incorporation — United Kingdom

4. **Title of the invention** — METHOD

5. **Name of your agent** *(if you have one)* — D YOUNG & CO

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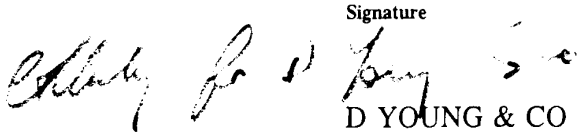

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12. Name and daytime telephone number of the person to contact in the United Kingdom

Dr C T Harding

023 8063 4816

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METHOD

FIELD OF INVENTION

The present invention relates to a method. In particular, the present invention relates to an assay method.

More in particular, the present invention relates to an assay method for identifying whether a candidate agent can modulate the binding of gp120 to CCR5.

BACKGROUND ART

It is desirable to identify agents that can modulate the interaction of CCR5 and gp120. By way of background information, gp120 is the envelope protein of HIV that is required for viral entry into the target cells and CCR5 is the cellular receptor to the β-chemokines RANTES, Mip-1-α and Mip-1-β. CCR5 has been identified as being an important receptor in HIV infection.

WO-A-98/00535 discloses a method for detecting gp120 binding on the surface of cells bearing a co-receptor. The method uses directly labelled protein. This method requires high affinity interactions (low nanomolar Kd). However, the affinity of gp120 proteins from different HIV strains varies. Hence, it is believed that this method may not be capable or even suitable to identify agents that can modulate the interaction of biologically relevant molecules.

WO-A-96/418884 discloses a method for screening for agents that may inhibit HIV-1 infection. That method utilises an antibody that, in itself, acts as an agent that would perturb CCR5 and gp120 binding. In addition the protein used in the specific examples cannot bind CCR5. Hence, the specific target receptors are not CCR5.

WO-A-97/37005 discloses an assay method for detecting and preventing HIV infection. The assay described detects fusion of cells expressing gp160 with cells expressing CD4 and CCR5. It is believed that this assay has inherent problems as containment and reagent provision issues would restrict the use of the method for high-throughput screening, particularly on a large scale.

US-A-5928881 discloses an assay that enables identification of CCR5 inhibitors. The assay requires directly labelled chemokine RANTES. We believe that this assay would not always be predictive of binding of gp120.

The present invention seeks to provide an effective assay method, readily adaptable to high throughput screening format, for identifying agents that would modulate medium to low affinity interactions of CCR5 with gp120.

SUMMARY ASPECTS OF THE PRESENT INVENTION

The present invention is based on the finding that *in vivo* the interaction of CCR5 with gp120 may be fairly weak. In this regard, our work with recombinant proteins suggests that the dissociation constant (Kd) of the interaction can be in the 10^{-7}M to 10^{-6}M range. This is contrast to the 10^{-9}M range described by others in model systems and in, for example, the patent applications cited above (e.g. WO-A-98/00535 and references within).

The assay method of the present invention utilises this finding. It enables detection of such weak interaction.

For some applications, the assay method of the present invention may be described as being a competitive binding study between a candidate agent and the gp120/CCR5 combination.

DETAILED ASPECTS OF THE PRESENT INVENTION

In one aspect, the present invention relates to an assay method for determining whether an agent is capable of modulating the interaction of CCR5 with gp120; the method comprising: incubating the agent with CCR5 and gp120 to form a first reaction mixture; and determining whether said agent modulates the interaction of CCR5 with gp120; wherein said gp120 is associated with CD4; and wherein said interaction is a low affinity binding.

In another aspect, the present invention relates to an agent identified by the method according to the present invention, wherein said agent is capable of modulating the interaction of CCR5 with gp120.

In a further aspect, the present invention relates to a process comprising the steps of: (a) performing the assay according to the present invention; (b) identifying one or more agents that are capable of modulating the interaction of CCR5 with gp120; and (c) preparing a quantity of those one or more identified agents.

In a further aspect, the present invention relates to a method of affecting the *in vivo* interaction of CCR5 with gp120 with an agent; wherein the agent is capable of modulating the interaction of CCR5 with gp120 in an *in vitro* assay method; wherein the *in vitro* assay method is the assay method according to the present invention.

In a further aspect, the present invention relates to the use of an agent in the preparation of a pharmaceutical composition for the treatment of a disease or condition associated with the interaction of CCR5 with gp120, wherein the agent is the agent according to the present invention and/or wherein the agent is capable of modulating the interaction of CCR5 with gp120 when assayed *in vitro* by the assay method according to the present invention.

In a further aspect, the present invention relates to a method of treating a subject with an agent, wherein the agent is the agent of the present invention and/or wherein the agent is capable of modulating the interaction of CCR5 with gp120 when assayed *in vitro* by the assay method according to the present invention.

For ease of reference, these and further aspects of the present invention are now discussed under appropriate section headings. However, the teachings under each section are not necessarily limited to each particular section.

PREFERABLE ASPECTS

Preferably the method includes the step of adding a ligand to said first reaction mixture to form a second reaction mixture; wherein said ligand is capable of indicating whether said agent has modulated said interaction.

Preferably said ligand has a detectable label.

Preferably said detectable label is a atom or a group capable of emitting fluorescent light.

Preferably said fluorescent atom is Eu^{3+}.

Preferably detection consists of enhancing natural fluorescence of the Eu^{3+} atom by addition of an enhancer solution known to the art.

Preferably said ligand comprises at least a first antibody.

Preferably said first antibody is capable of binding to gp120; and wherein said binding is high affinity binding.

Preferably said first antibody is associated with a detectable label.

Preferably said ligand may comprise a second antibody.

Preferably said second antibody is capable of binding to said first antibody.

Preferably said second antibody is an anti-IgG antibody.

Preferably said detectable label is associated with said second antibody.

Preferably, the agent is capable of adversely modulating the weak binding interaction of CCR5 and gp120.

Examples of adverse modulation include the separation of, the prevention of the binding of, the cleavage of any one or more of, CCR5 and gp120 – and/or changing their folding configuration so that one or more of gp120 and CCR5 is inoperative.

Further examples of adverse modulation include the separation of, the prevention of the binding of, the cleavage of any one or more of, gp120 and CD4 – and/or changing their folding configuration so that one or more of gp120 and CD4 is inoperative.

ADVANTAGES

The present invention has a number of advantages. These advantages will be apparent in the following description.

By way of example, the present invention is advantageous since it provides a commercially useful assay to identify suitable agents that could be used *in vivo* to treat conditions associated with CCR5/gp120 binding.

By way of further example, the present invention is advantageous since the method detects interaction of gp120 with cells that express CCR5 alone.

By way of example, the present invention is advantageous since the method is amenable to high throughput screening (HTS).

AMINO ACID SEQUENCE

As used herein, the term "amino acid sequence" is synonymous with the term "polypeptide" and/or the term "protein". In some instances, the term "amino acid sequence" is synonymous with the term "peptide". In some instances, the term "amino acid sequence" is synonymous with the term "protein".

The amino acid sequence may be prepared isolated from a suitable source, or it may be made synthetically or it may be prepared by use of recombinant DNA techniques.

In one aspect, the present invention provides an amino acid sequence that is capable of acting as a target in an assay for the identification of one or more agents and/or derivatives thereof capable of affecting gp120/CCR5 binding.

NUCLEOTIDE SEQUENCE

As used herein, the term "nucleotide sequence" is synonymous with the term "polynucleotide".

The nucleotide sequence may be DNA or RNA of genomic or synthetic or of recombinant origin. The nucleotide sequence may be double-stranded or single-stranded whether representing the sense or antisense strand or combinations thereof.

For some applications, preferably, the nucleotide sequence is DNA.

For some applications, preferably, the nucleotide sequence is prepared by use of recombinant DNA techniques (e.g. recombinant DNA).

For some applications, preferably, the nucleotide sequence is cDNA.

For some applications, preferably, the nucleotide sequence may be the same as the naturally occurring form for this aspect.

In one aspect, the present invention provides a nucleotide sequence encoding a substance capable of acting as a target in an assay (such as a yeast two hybrid assay) for the identification of one or more agents and/or derivatives thereof capable of affecting the substance in order to modulate CCR5/gp120 binding.

VARIANTS/HOMOLOGUES/DERIVATIVES

In addition to the specific amino acid sequences and nucleotide sequences mentioned herein, the present invention also encompasses the use of variants, homologue and derivatives thereof. Here, the term "homology" can be equated with "identity".

In the present context, an homologous sequence is taken to include an amino acid sequence which may be at least 75, 85 or 90% identical, preferably at least 95 or 98% identical. In particular, homology should typically be considered with respect to those regions of the sequence (such as amino acids at positions 51, 56 and 57) known to be essential for an activity. Although homology can also be considered in terms of similarity (i.e. amino acid residues having similar chemical properties/functions), in the context of the present invention it is preferred to express homology in terms of sequence identity.

Homology comparisons can be conducted by eye, or more usually, with the aid of readily available sequence comparison programs. These commercially available computer programs can calculate % homology between two or more sequences.

% homology may be calculated over contiguous sequences, i.e. one sequence is aligned with the other sequence and each amino acid in one sequence is directly compared with the corresponding amino acid in the other sequence, one residue at a time. This is called an "ungapped" alignment. Typically, such ungapped alignments are performed only over a relatively short number of residues.

Although this is a very simple and consistent method, it fails to take into consideration that, for example, in an otherwise identical pair of sequences, one insertion or deletion will cause the following amino acid residues to be put out of alignment, thus potentially resulting in a large reduction in % homology when a global alignment is performed. Consequently, most sequence comparison methods are designed to produce optimal alignments that take into consideration possible insertions and deletions without penalising unduly the overall homology score. This is achieved by inserting "gaps" in the sequence alignment to try to maximise local homology.

However, these more complex methods assign "gap penalties" to each gap that occurs in the alignment so that, for the same number of identical amino acids, a sequence alignment with as few gaps as possible - reflecting higher relatedness between the two compared sequences - will achieve a higher score than one with many gaps. "Affine gap costs" are typically used that charge a relatively high cost for the existence of a gap and a smaller penalty for each subsequent residue in the gap. This is the most commonly used gap scoring system. High gap penalties will of course produce optimised alignments with fewer gaps. Most alignment programs allow the gap penalties to be modified. However, it is preferred to use the default values when using such software for sequence comparisons. For example when using the GCG Wisconsin Bestfit package (see below) the default gap penalty for amino acid sequences is -12 for a gap and -4 for each extension.

Calculation of maximum % homology therefore firstly requires the production of an optimal alignment, taking into consideration gap penalties. A suitable computer program for carrying out such an alignment is the GCG Wisconsin Bestfit package

(University of Wisconsin, U.S.A.; Devereux et al., 1984, Nucleic Acids Research 12:387). Examples of other software than can perform sequence comparisons include, but are not limited to, the BLAST package (see Ausubel et al., 1999 ibid – Chapter 18), FASTA (Atschul et al., 1990, J. Mol. Biol., 403-410) and the GENEWORKS suite of comparison tools. Both BLAST and FASTA are available for offline and online searching (see Ausubel et al., 1999 ibid, pages 7-58 to 7-60). However it is preferred to use the GCG Bestfit program.

Although the final % homology can be measured in terms of identity, the alignment process itself is typically not based on an all-or-nothing pair comparison. Instead, a scaled similarity score matrix is generally used that assigns scores to each pairwise comparison based on chemical similarity or evolutionary distance. An example of such a matrix commonly used is the BLOSUM62 matrix - the default matrix for the BLAST suite of programs. GCG Wisconsin programs generally use either the public default values or a custom symbol comparison table if supplied (see user manual for further details). It is preferred to use the public default values for the GCG package, or in the case of other software, the default matrix, such as BLOSUM62.

Once the software has produced an optimal alignment, it is possible to calculate % homology, preferably % sequence identity. The software typically does this as part of the sequence comparison and generates a numerical result.

The sequences may also have deletions, insertions or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent substance. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues as long as the secondary binding activity of the substance is retained. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine, valine, glycine, alanine, asparagine, glutamine, serine, threonine, phenylalanine, and tyrosine.

Conservative substitutions may be made, for example according to the Table below. Amino acids in the same block in the second column and preferably in the same line in the third column may be substituted for each other:

ALIPHATIC	Non-polar	G A P
		I L V
	Polar – uncharged	C S T M
		N Q
	Polar – charged	D E
		K R
AROMATIC		H F W Y

EXPRESSION VECTORS

The nucleotide sequence for use as the target or for expressing the target can be incorporated into a recombinant replicable vector. The vector may be used to replicate and express the nucleotide sequence in and/or from a compatible host cell. Expression may be controlled using control sequences which include promoters/enhancers and other expression regulation signals. Prokaryotic promoters and promoters functional in eukaryotic cells may be used. Tissue specific or stimuli specific promoters may be used. Chimeric promoters may also be used comprising sequence elements from two or more different promoters described above.

The protein produced by a host recombinant cell by expression of the nucleotide sequence may be secreted or may be contained intracellularly depending on the sequence and/or the vector used. The coding sequences can be designed with signal sequences which direct secretion of the substance coding sequences through a particular prokaryotic or eukaryotic cell membrane.

FUSION PROTEINS

The target amino acid sequence may be produced as a fusion protein, for example to aid in extraction and purification. Examples of fusion protein partners include glutathione-S-transferase (GST), 6xHis, GAL4 (DNA binding and/or transcriptional activation domains) and (-galactosidase. It may also be convenient to include a proteolytic cleavage site between the fusion protein partner and the protein sequence of interest to allow removal of fusion protein sequences. Preferably the fusion protein will not hinder the activity of the target.

The fusion protein may comprise an antigen or an antigenic determinant fused to the substance of the present invention. In this embodiment, the fusion protein may be a non-naturally occurring fusion protein comprising a substance which may act as an adjuvant in the sense of providing a generalised stimulation of the immune system. The antigen or antigenic determinant may be attached to either the amino or carboxy terminus of the substance.

In another embodiment of the invention, the amino acid sequence may be ligated to a heterologous sequence to encode a fusion protein. For example, for screening of peptide libraries for agents capable of affecting the substance activity, it may be useful to encode a chimeric substance expressing a heterologous epitope that is recognized by a commercially available antibody.

CCR5

An essential component of the assay is CCR5.

CCR5 is a chemokine receptor that typically is present on the surfaces of certain cells within the human body.

The term "chemokine", is a contraction of "chemotactic cytokines". The chemokines comprise a large family of proteins which have in common important structural features and which have the ability to attract leukocytes. As leukocyte chemotactic factors, chemokines play an indispensable role in the attraction of leukocytes to various tissues of the body, a process which is essential for both inflammation and the body's response to infection. Because chemokines and their receptors are central to the pathophysiology of inflammatory and infectious diseases, agents which are active in modulating, preferably antagonizing, the activity of chemokines and their receptors, are useful in the therapeutic treatment of such inflammatory and infectious diseases.

The chemokine receptor CCR5 is of particular importance in the context of treating inflammatory and infectious diseases. CCR5 is a receptor for chemokines, especially for the macrophage inflammatory proteins (MIP) designated MIP-1α and MIP-1β, and for a protein which is regulated upon activation and is normal T-cell expressed and secreted (RANTES).

Background teachings on CCR5 may be found in WO-A-97/32019.

The nucleotide sequence encoding same and the amino acid sequence for same are presented in the attached sequence listings.

Background teachings on CCR5 have also been presented by Victor A. McKusick *et al* on http://www.ncbi.nlm.nih.gov/Omim. The following information concerning CCR5 has been extracted from that source.

"Samson et al. (1996) cloned a human C-C chemokine receptor gene from a human genomic DNA library based on its similarity to a murine C-C chemokine receptor clone (MOP020). The human gene, which they designated ChemR13, encodes a 352-amino acid protein (designated CCCKR5 by them) with a calculated molecular mass of 40,600 Da and a potential N-linked glycosylation site. With a set of overlapping lambda clones, they showed that the gene is 17.5 kb from the CMKBR2 gene. The 2 coding regions share 75% DNA and amino acid sequence identity.

Samson et al. (1996) functionally expressed the gene in a stably transfected CHO-K1 cell line. In transfected cells, macrophage inflammatory protein (MIP)-1-α appeared to be the most potent agonist for CCCKR5, with MIP-1-β and RANTES also active at physiologic concentrations. Samson et al. (1996) detected transcript from the gene in a promyeloblastic cell line, which suggested a potential role for the chemokine receptor in granulocyte lineage proliferation and differentiation.

By radiation hybrid mapping, Liu et al. (1996) localized the CCR5 gene (designated CKR5 by them) to chromosome 3p21.

The C-C chemokine receptor CMKBR5 was identified as a coreceptor for the human immunodeficiency virus-1 (HIV-1) by Deng et al. (1996) and Dragic et al. (1996). CMKBR5 and fusin (162643) facilitate the fusion of HIV-1 with the plasma membrane of CD4(+) cells (CD4; 186940). Deng et al. (1996) found that CMKBR5, and not fusin, promotes entry of the macrophage-tropic viruses believed to be the key pathogenic strains in vivo. Dragic et al. (1996) showed that MIP-1-α, MIP-1-β, and RANTES each inhibit infection of CD4(+) cells by primary, nonsyncytium-inducing (NSI) HIV-1 strains at the virus entry stage and also block env-mediated cell-cell fusion. Both groups showed that expression of the CCCKR5 protein renders nonpermissive CD4(+) cells susceptible to infection by HIV-1 strains. Alkhatib et al. (1996) reported similar observations and detected mRNA for the receptor only in cell types susceptible to macrophage-tropic isolates of HIV-1. See also Choe et al. (1996), who implicated both CCR5 and CCR3 in the ability of HIV-1 to infect cells expressing those receptors.

Some individuals remain uninfected by HIV-1 despite repeated exposure to the virus. Both

Liu et al. (1996) and Samson et al. (1996) identified a molecular basis for such HIV-1 resistance. Samson et al. (1996) postulated that variants of the CMKBR5 gene may be responsible for relative or absolute resistance to HIV-1 infection. In an HIV-1-infected patient with slow disease progression, Samson et al. (1996) identified a heterozygous 32-bp deletion in the CMKBR5 gene that results in a frameshift and premature termination of translation of the transcript. Liu et al. (1996) identified the same homozygous 32-bp deletion with CMKBR5 in 2 individuals who, though multiply exposed to HIV-1 infection, remained uninfected. Liu et al. (1996) found that the deletion comprises nucleotides 794 to 825 of the cDNA sequence and results in a reading frameshift after amino acid 174, inclusion of 7 novel amino acids, and truncation at codon 182. They showed that the severely truncated protein could not be detected at the surface of cells that normally express the protein. Samson et al. (1996) stated that the mutant protein lacks the last 3 of 7 putative transmembrane regions of the receptor as well as regions involved in G protein coupling and signal transduction. Through in vitro fusion assays, both Liu et al. (1996) and Samson et al. (1996) determined that the truncated receptor did not allow fusion of CD4(+) cells with cells expressing env protein from either macrophage-tropic or dual-tropic viruses. Samson et al. (1996) found that coexpression of the deletion mutant with wildtype CCR5 reduced the fusion efficiency of 2 different viral envelope proteins in 3 independent experiments.

Dean et al. (1996) reported results of their CKR5 studies in 1,955 individuals included in 6 well-characterized AIDS cohort studies. They identified 17 individuals who were homozygous for the CKR5 32-bp deletion allele (601373.0001). Deletion homozygotes occurred exclusively among the 612 members of the HIV-1-exposed, antibody-negative group and not at all in 1,343 HIV-1 infected individuals. The frequency of the CKR5 deletion heterozygotes was significantly elevated in groups of individuals who had survived HIV-1 infection for more than 10 years. In some risk groups the frequency of CKR5 deletion heterozygotes was twice as frequent as in groups with rapid progressors to AIDS. Survival analysis clearly showed that the disease progression was slower in CKR5 deletion heterozygotes than in individuals homozygous for the normal CKR5 allele. Dean et al. (1996) postulated that the CKR5 32-bp deletion may act as 'a recessive restriction gene against HIV-1 infection' and may exert a dominant phenotype of delayed progression to AIDS among infected individuals. Dean et al. (1996) reported that in addition to the CKR5 32-bp deletion allele, they found unique single-strand conformation polymorphisms (SSCPs) in other patients, some of whom were long-term nonprogressors. They speculated that at least some of these alleles disrupt CKR5 function and inhibit the spread of HIV-1 or the progression to AIDS. Dean et al. (1996) recommended that the entire coding region of CKR5 be screened in nonprogressors and in rapid progressors to identify other CKR5 variants.

To determine the role of the 32-bp deletion in CKR5 in HIV-1 transmission and disease progression, Huang et al. (1996) analyzed the CKR5 genotype of 1,252 homosexual men enrolled in the Chicago component of the Multicenter AIDS Cohort Study. No infected participant was found to be homozygous for the 32-bp deletion allele, whereas 3.6% of at-risk

but uninfected Caucasian participants were homozygous, showing the highly protective role of this genotype against sexual acquisition of HIV-1. No evidence was found that suggested heterozygotes were protected against HIV-1 infection, but a limited protective role against disease progression was noted.

Zimmerman et al. (1997) reported results of a large study that analyzed the frequency of the 32-bp deletion allele of CMKBR5 in populations from North America, Asia, and Africa. Ansari-Lari et al. (1997) also published data on the population frequencies of various mutations in CCR5. The study indicated that the mutations are relatively specific to different ethnicities; apart from the 32-bp deletion allele in the American Caucasian population, and 2 alleles in Chinese and Japanese populations, the CCR5 locus did not show a high degree of genetic variation. The authors stated that, while additional population screening at this locus might identify other sequence variants, their frequencies are likely to be less than 0.01. The frequency of the 32-bp deletion allele in American Caucasians was approximately 0.16, a value somewhat higher than that previously reported for this group.

Smith et al. (1997) analyzed 2-locus genotypes and found that the 32-bp deletion at the CCR5 locus and the 64I allele at the CCR2 locus are in strong, perhaps complete, linkage disequilibrium with each other. This means that CCR5-del32 invariably occurs on a chromosome with allele CCR2-64V, whereas CCR2-64I occurs on a chromosome that has the wildtype (undeleted) allele at the CCR5 locus. Thus, they could estimate the independent effects of the CCR2 and CCR5 polymorphisms. An estimated 38 to 45% of AIDS patients who had rapid progression of less than 3 years from HIV-1 exposure to onset of AIDS symptoms could be attributed to their wildtype status at one or the other of these loci, whereas the survival of 28 to 29% of long-term survivors, who avoided AIDS for 16 years or more, could be explained by a mutant genotype for CCR2 or CCR5.

Biti et al. (1997) reported an HIV-infected, asymptomatic individual of European descent who was found to be homozygous for the 32-bp deletion. The presence of homozygosity was supported by genotyping his sole surviving parent (a heterozygote) and his sibs (a CCR5-del32 homozygous brother, a heterozygous brother, and a CCR5 wildtype homozygous sister). The patient presented in 1992 with a seroconversion-like illness of 1-month duration, at which time he was diagnosed HIV-1 seropositive by Western blot. At the time of their report, his CD4+ T-cell count was 460, and a plasma RNA viral load test showed 19,000 copies per milliliter. Biti et al. (1997) noted that the tropism of the infecting HIV-1 strain was still under investigation.

Using a panel of monoclonal antibodies specific for human CCR5, Rottman et al. (1997) showed by immunohistochemistry and flow cytometry that CCR5 is expressed by bone marrow-derived cells known to be targets for HIV-1 infection, including a subpopulation of lymphocytes and monocytes/macrophages in blood, primary and secondary lymphoid organs, and noninflamed tissues. In the central nervous system, CCR5 was expressed on neurons, astrocytes, and microglia. In other tissues, CCR5 was expressed on epithelium, endothelium, vascular smooth muscle, and fibroblasts. Chronically inflamed tissues contained an increased

number of CCR5-positive mononuclear cells, and the number of immunoreactive cells was directly associated with a histopathologic correlate of inflammatory severity. The results suggested that CCR5-positive cells are recruited to inflammatory sites and, as such, may facilitate transmission of macrophagetropic strains of HIV-1.

Cohen et al. (1997) studied a cohort of 33 HIV-1 nonprogressors and compared 21 patients who were homozygous wildtype at the CCR5 locus with 12 who were heterozygous for the 32-bp deletion mutation in CCR5. There were no differences in CD4+ or CD8+ T-cell counts, or in plasma or lymph node viral loads. The authors concluded that CCR5 is not the sole determinant of long-term nonprogression in some HIV-1 infected individuals. Although no differences were detected at an average of 11 years postinfection, the authors suggest that CCR5 may still play a role in nonprogression by limiting viral replication during acute infection.

Although CD4 was identified initially as the cellular receptor for HIV, several lines of evidence indicated that expression of CD4 alone was insufficient to confer susceptibility to infection by the virus. Specifically, HIV did not infect mouse cells transfected with a human CD4 expression vector or mice transgenic for the expression of human CD4. Furthermore, although HIV binding and internalization can be mediated by CD4 acting together with one of several members of the chemokine receptor superfamily, CCR5 appears to be the critical coreceptor used by HIV in the initial stages of infection. However, because mouse CCR5 differs significantly from human CCR5, it cannot function as a coreceptor for HIV, and thus, expression of human CD4 alone is insufficient to permit entry of HIV into mouse cells. Browning et al. (1997) found that mice transgenic for both CD4 and CCR5 are susceptible to HIV infection.

Zagury et al. (1998) found that there were factors other than the CCR5 polymorphism accounting for the fact that exposure to HIV-1 does not usually lead to infection. Although this fact could be because of insufficient virus titer, there is abundant evidence that some individuals resist infection even when directly exposed to a high titer of HIV. This protection is related to homozygous mutations in CCR5, the receptor for the β-chemokines, and earlier studies had shown that the same chemokines markedly suppressed the nonsyncytial inducing variants of HIV-1, the chief virus type transmitted from person to person. However, CCR5 mutations are not likely to be the unique mechanism of protection because HIV-1 variants can use other chemokine receptors as their coreceptor and, indeed, infection has been demonstrated within the presence of such mutations. Zagury et al. (1998) found transient natural resistance over time of most of 128 hemophiliacs who were inoculated repeatedly with HIV-1-contaminated factor VIII concentrate from plasma during 1980 to 1985, before the development of the HIV blood test. Furthermore, and remarkably, 14 subjects remained unaffected to the time of the report, and in these subjects homozygous CCR5 mutations were found in none, but in most of them there was overproduction of β-chemokines. In vitro experiments confirmed the potent anti-HIV suppressive effect of these chemokines. The chemokines studied were generically referred to as MMR, an abbreviation for MIP-1-α, MIP-

1-β, and RANTES.

Martin et al. (1998) showed by genetic association analysis of 5 cohorts of people with AIDS that infected individuals homozygous for a multisite haplotype of the CCR5 regulatory region containing the promoter allele, CCR5P1, progress to AIDS more rapidly than those with other CCR5 promoter genotypes, particularly in the early years after infection. Composite genetic epidemiologic analyses of the genotypes bearing CCR5P1, CCR5-delta-32, CCR2-64I (see 601267), and SDF1-3-prime A (see 600835) affirmed distinct regulatory influences for each gene on AIDS progression. An estimated 10 to 17% of patients who developed AIDS within 3.5 years of HIV-1 infection did so because they were homozygous for CCR5P1/P1, and 7 to 13% of all people carry this susceptible genotype. The cumulative and interactive influence of these AIDS restriction genes illustrates the multigenic nature of host factors limiting AIDS disease progression.

CCR5 and CCR2 are tightly linked on 3p22-p21, separated by 20 kb. Common allelic variants in both genes are associated with slower progression to AIDS after infection. The protective influences of CCR5-delta-32 and CCR2-64I are independent in AIDS cohorts, and the 2 mutations have never been found on the same chromosome haplotype. The physical proximity of CCR2 and CCR5, the equivalent functional efficiency of alternative CCR2 allelic products as chemokine or HIV-1 coreceptors, and the rarity of HIV-1 strains that use the CCR2 receptor led to the speculation that CCR2-64I may be hitchhiking (or tracking by linkage disequilibrium) with an undiscovered CCR5 variant, perhaps in the cis-regulatory region, that is directly responsible for the CCR2-64I protective effect. Martin et al. (1998) found that among 2,603 individuals enrolled in 5 AIDS cohorts, CCR2-64I was always found on a CCR5P1-bearing haplotype and that CCR5-delta-32 was consistently found on a CCR5P1 haplotype as well. All CCR2-64I/64I homozygotes were always CCR5P1/P1 homozygotes (N = 43). Similarly, all CCR5-delta-32/delta-32 homozygotes were CCR5P1/P1 homozygotes (N = 18). Finally, none of 657 individuals who lacked the CCR5P1 allele had either the CCR5-delta-32 or the CCR2-64I allele. Thus the entire CCR2-CCR5 complex can be considered as a 6-allele genotype system, based on the composite CCR2 and CCR5 haplotype.

In a denaturing high-pressure liquid chromatography (DHPLC) screen of AIDS patients, Martin et al. (1998) detected 4 common allelic variants (CCR5P1-P4); 6 rare alleles (CCR5P5-P10) were discovered as heterozygotes upon subsequent single-strand conformation polymorphism (SSCP) screening of 5 AIDS cohorts. Sequence analysis of the CCR5 promoter region of individuals homozygous for the CCR5P1-P4 variants and heterozygotes of the 6 rare variants revealed 10 polymorphic nucleotide positions that described 10 CCR5 promoter haplotype alleles, referred to as promoter alleles. McDermott et al. (1998) reported a G/T variant, corresponding to position 303 of the promoter region, that showed an epidemiologic association with rapid progression to AIDS.

Farzan et al. (1999) showed that the chemokine receptor CCR5, a principal HIV-1 coreceptor, is posttranslationally modified by O-linked glycosylation and by sulfation of its N-terminal tyrosines. Sulfated tyrosines contributed to the binding of CCR5 to MIP-1-α, MIP-1-β, and

HIV-1 gp120/CD4 complexes, and to the ability of HIV-1 to enter cells expressing CCR5 and CD4. Farzan et al. (1999) concluded that tyrosine sulfation may contribute to the natural function of many 7-transmembrane-segment receptors and may be a modification common to primate immunodeficiency virus coreceptors.

Carrington et al. (1999) reviewed the growing number of genetic variants within the coding and 5-prime regulatory region of CCR5 that had been identified, several of which have functional consequences for HIV-1 pathogenesis. The findings provided logic for the development of therapeutic strategies that target the interaction of HIV-1 envelope and CCR5 in HIV-1 associated disease."

gp120

An essential component of the assay is gp120. Information on gp120 has been presented above. Further teachings on gp120 may be found in the art, such as in some of the patent applications cited above.

The nucleotide sequence encoding gp120 and the amino acid sequence for same are presented in the attached sequence listings. .

CD4

In the assay of the present invention the gp120 is typically associated with CD4. A suitable CD4 is a commercialy available soluble version of CD4 (baculovirus expressed sCD4). In the preparation of the first reaction mixture, the gp120 and the CD4 may be added sequentially, simultaneously or together.

Background teachings on CD4 are mentioned above.

Background teachings on CD4 may also be found in WO-A-89/03222. The nucleotide sequence encoding same and the amino acid sequence of same is presented in the attached sequence listings.

In addition, further background teachings on CD4 have been presented by Victor A. McKusick *et al* on http://www.ncbi.nlm.nih.gov/Omim. The following further information concerning CD4 has been extracted from that source.

"Circulating mature T lymphocytes constitute a heterogeneous cell population with 2 major phenotypes, one expressing the CD4 marker on its surface (generally associated with helper/inducer function), and the other expressing the CD8 antigen (usually associated with cytotoxic/suppressor activity). Amadori et al. (1995) noted that evaluation of the CD4/CD8 ratio is routine in AIDS patients for the assessment of immune function and added that not only HIV infection but also other acute viral diseases, such as infections by cytomegalovirus, Epstein-Barr virus, and influenza virus, are usually associated with an inversion of the CD4/CD8 ratio. A low ratio is also a hallmark of intense, chronic immune responses, such as allograft rejection and graft-versus-host disease. CD4/CD8 ratios of 1.5 to 2.5 are usually considered normal. The occasional finding of a CD4/DC8 ratio less than 1 in otherwise normal, healthy individuals is usually disregarded.

Because of a personal interest on the part of one member of the group (A.A.) who had shown an invariable ratio less than 1 over many years, and because the ratio in mice was shown by Kraal et al. (1983) to be under genetic control, Amadori et al. (1995) studied the genetic pattern of inheritance of the ratio in a population of 468 healthy blood donors. Distribution of the ratio in males and females was significantly different and was significantly affected by age. In 46 randomly selected families, the parental CD4/CD8 ratios significantly influenced the ratio in offspring. Complex segregation analysis of the data rejected the non-genetic hypothesis; among the genetic models tested, a major recessive gene with a polygenic component and random environmental effects was the most parsimonious model. Overall, Amadori et al. (1995) found that 57% of the variation in the CD4/CD8 ratio could be attributed to genetic factors, as opposed to noninherited (stochastic or environmental) factors. In mice, the CD4/CD8 ratio appears to be under the genetic control of a single dominant gene (Kraal et al., 1983). Chakravarti (1995) pointed to several important implications of the simple observation in this study. First, norms for the ratio must be defined separately for different ages, genders, and perhaps even populations. The possibility of a major gene determining the ratio implies that family history of low ratios may need to be considered for accurate prognosis of HIV or other infection. The identification of genes underlying this phenotype will lead to a better understanding of the mechanisms that commit immature thymocytes to the helper or cytotoxic lineages, and the site in the developmental chain in which they function. Lastly, identification of the genes will also help answer questions regarding genetic factors that control infection and immunity.

To define the mode of inheritance of the CD4/CD8 ratio, Clementi et al. (1999) examined the absolute number of CD4 and CD8 cells in a large unselected control population and in members of 70 nuclear families. Pedigrees of nuclear families were analyzed by complex segregation analysis. Data were adjusted before this analysis to remove the effects of relevant covariates. The nongenetic transmission and the multifactorial model could be easily rejected for both CD4 and CD8 cells. The best fitting models were a major autosomal recessive gene with a residual multifactorial effect controlling the high number of CD4 and a major autosomal recessive gene with a residual multifactorial effect controlling the high number of

CD8 cells. The authors pointed out that the knowledge of the CD4+ cell number and the proportion between CD4+ and CD8+ T cells could be a useful parameter in predicting human immunodeficiency virus infection outcome."

LOW AFFINITY BINDING

As used in relation to the present invention, preferably the term "low affinity binding" means a Kd value of at least about 200 nM. More preferably the term "low affinity binding" means a Kd value of more than about 200 nM.

LIGAND

In a preferred aspect, the assay method of the present invention utilises a ligand. This ligand is capable of indicating whether said agent has modulated said interaction.

The ligand may be any suitable ligand. The ligand may be an organic chemical compound. The ligand may be a protein.

In a preferred aspect, the ligand carries a detectable label. This detectable label may become detectable once modulation has occurred – such as through a change in spectrometric properties of the ligand.

This label may be detectable by, for example, spectrometric means – such as spectrophotometric means. Preferably, however, the detectable label is a fluorescent label. Here, any suitable fluorescent can be used.

In a preferred aspect, the ligand comprises at least one antibody, preferably at least two antibodies. In this respect, the at least one antibody is termed a "first antibody". This first antibody may be able to bind to any of the agent to gp120. In a preferred aspect, the first antibody is able to bind to gp120 with high affinity and specificity, and in a mode that does not disturb the interaction of gp120 with either CD4 or CCR5.

If the ligand comprises at least two antibodies, then the at least one other antibody is called a "second antibody". The second antibody may be able to bind the first antibody. In a preferred aspect, the second antibody is able to bind to the first

antibody with high affinity and specificity, and in a way that does not disturb the interaction of the first antibody with its ligand.

Preferably, the ligand comprises the first antibody and the second antibody. In this embodiment, in the preparation of the second reaction mixture the first antibody and the second antibody may be added sequentially, simultaneously or together.

Preferably the second antibody carries the detectable label. If there is no second antibody, the first antibody typically carries the detectable label.

ANTIBODY

As indicated, preferably the ligand for use in the assay method of the present invention comprises one or two antibodies.

The "antibody" as used herein includes but is not limited to, polyclonal, monoclonal, chimeric, single chain, Fab fragments and fragments produced by a Fab expression library. Such fragments include fragments of whole antibodies which retain their binding activity for a target substance, Fv, F(ab') and F(ab')2 fragments, as well as single chain antibodies (scFv), fusion proteins and other synthetic proteins which comprise the antigen-binding site of the antibody. Furthermore, the antibodies and fragments thereof may be humanised antibodies, for example as described in US-A-239400. Neutralizing antibodies, i.e., those which inhibit biological activity of the substance polypeptides, are especially preferred for diagnostics and therapeutics.

Antibodies may be produced by standard techniques, such as by immunisation with the substance of the invention or by using a phage display library.

If polyclonal antibodies are desired, a selected mammal (e.g., mouse, rabbit, goat, horse, etc.) is immunised with an immunogenic polypeptide bearing a epitope(s) obtainable from an identified agent and/or substance of the present invention. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include, but are not limited to, Freund's, mineral gels such as aluminium hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol. BCG (Bacilli Calmette-Guerin) and Corynebacterium parvum are potentially useful human adjuvants which may be employed if purified the

substance polypeptide is administered to immunologically compromised individuals for the purpose of stimulating systemic defence.

Serum from the immunised animal is collected and treated according to known procedures. If serum containing polyclonal antibodies to an epitope obtainable from an identifed agent and/or substance of the present invention contains antibodies to other antigens, the polyclonal antibodies can be purified by immunoaffinity chromatography. Techniques for producing and processing polyclonal antisera are known in the art. In order that such antibodies may be made, the invention also provides polypeptides of the invention or fragments thereof haptenised to another polypeptide for use as immunogens in animals or humans.

Monoclonal antibodies directed against particular epitopes can also be readily produced by one skilled in the art. The general methodology for making monoclonal antibodies by hybridomas is well known. Immortal antibody-producing cell lines can be created by cell fusion, and also by other techniques such as direct transformation of B lymphocytes with oncogenic DNA, or transfection with Epstein-Barr virus. Panels of monoclonal antibodies produced against orbit epitopes can be screened for various properties; i.e., for isotype and epitope affinity.

Monoclonal antibodies may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique originally described by Koehler and Milstein (1975 Nature 256:495-497), the human B-cell hybridoma technique (Kosbor et al (1983) Immunol Today 4:72; Cote et al (1983) Proc Natl Acad Sci 80:2026-2030) and the EBV-hybridoma technique (Cole et al (1985) Monoclonal Antibodies and Cancer Therapy, Alan R Liss Inc, pp 77-96). In addition, techniques developed for the production of "chimeric antibodies", the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity can be used (Morrison et al (1984) Proc Natl Acad Sci 81:6851-6855; Neuberger et al (1984) Nature 312:604-608; Takeda et al (1985) Nature 314:452-454). Alternatively, techniques described for the production of single chain antibodies (US Patent No. 4,946,779) can be adapted to produce the substance specific single chain antibodies.

Antibodies may also be produced by inducing in vivo production in the lymphocyte population or by screening recombinant immunoglobulin libraries or panels of highly specific binding reagents as disclosed in Orlandi et al (1989, Proc Natl Acad Sci 86: 3833-3837), and Winter G and Milstein C (1991; Nature 349:293-299).

Antibody fragments which contain specific binding sites for the substance may also be generated. For example, such fragments include, but are not limited to, the F(ab')2 fragments which can be produced by pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the F(ab')2 fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity (Huse WD et al (1989) Science 256:1275-128 1).

ASSAY

Any one or more of appropriate targets - such as an amino acid sequence and/or nucleotide sequence - may be used for identifying an agent capable of modulating the interaction of gp120 with CCR5 in any of a variety of drug screening techniques. The target employed in such a test may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The abolition of target activity or the formation of binding complexes between the target and the agent being tested may be measured.

The assay of the present invention may be a screen, whereby a number of agents are tested. In one aspect, the assay method of the present invention is a high through put screen.

Techniques for drug screening may be based on the method described in Geysen, European Patent Application 84/03564, published on September 13, 1984. In summary, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The peptide test compounds are reacted with a suitable target or fragment thereof and washed. Bound entities are then detected - such as by appropriately adapting methods well known in the art. A purified target can also be coated directly onto plates for use in a drug screening techniques. Alternatively, non-neutralising antibodies can be used to capture the peptide and immobilise it on a solid support.

This invention also contemplates the use of competitive drug screening assays in which neutralising antibodies capable of binding a target specifically compete with a test compound for binding to a target.

Another technique for screening provides for high throughput screening (HTS) of agents having suitable binding affinity to the substances and is based upon the method described in detail in WO-A-84/03564.

It is expected that the assay methods of the present invention will be suitable for both small and large-scale screening of test compounds as well as in quantitative assays.

In one preferred aspect, the present invention relates to a method of identifying agents that selectively modulate the interaction between gp120 and CCR5.

In a preferred aspect, the assay of the present invention utilises cells that display CCR5 on their surface. These cells may be isolated from a subject possessing such cells. However, preferably, the cells are prepared by transfecting cells so that upon transfect those cells display on their surface CCR5. Teachings for preparing such transfected cells may be found in US-A-5939320.

REPORTERS

A wide variety of reporters may be used in the assay methods (as well as screens) of the present invention with preferred reporters providing conveniently detectable signals (eg. by spectroscopy). By way of example, a reporter gene may encode an enzyme which catalyses a reaction which alters light absorption properties.

Other protocols include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA) and fluorescent activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilising monoclonal antibodies reactive to two non-interfering epitopes may even be used. These and other assays are described, among other places, in Hampton R et al (1990, Serological Methods, A Laboratory Manual, APS Press, St Paul MN) and Maddox DE et al (1983, J Exp Med 15 8:121 1).

Examples of reporter molecules include but are not limited to (galactosidase, invertase, green fluorescent protein, luciferase, chloramphenicol, acetyltransferase, (glucuronidase, exo-glucanase and glucoamylase. Alternatively, radiolabelled or fluorescent tag-labelled nucleotides can be incorporated into nascent transcripts which are then identified when bound to oligonucleotide probes.

By way of further examples, a number of companies such as Pharmacia Biotech (Piscataway, NJ), Promega (Madison, WI), and US Biochemical Corp (Cleveland, OH) supply commercial kits and protocols for assay procedures. Suitable reporter molecules or labels include those radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents as well as substrates, cofactors, inhibitors, magnetic particles and the like. Patents teaching the use of such labels include US-A-3817837; US-A-3850752; US-A-3939350; US-A-3996345; US-A-4277437; US-A-4275149 and US-A-4366241.

HOST CELLS

Polynucleotides for use in the present invention – such as for use as targets or for expressing targets - may be introduced into host cells.

The term "host cell" - in relation to the present invention includes any cell that could comprise the target for the agent of the present invention.

Here, polynucleotides may be introduced into prokaryotic cells or eukaryotic cells, for example yeast, insect or mammalian cells.

Polynucleotides of the invention may introduced into suitable host cells using a variety of techniques known in the art, such as transfection, transformation and electroporation. Where polynucleotides of the invention are to be administered to animals, several techniques are known in the art, for example infection with recombinant viral vectors such as retroviruses, herpes simplex viruses and adenoviruses, direct injection of nucleic acids and biolistic transformation.

Thus, a further embodiment of the present invention provides host cells transformed or transfected with a polynucleotide that is or expresses the target of the present invention. Preferably said polynucleotide is carried in a vector for the replication and

expression of polynucleotides that are to be the target or are to express the target. The cells will be chosen to be compatible with the said vector and may for example be prokaryotic (for example bacterial), fungal, yeast or plant cells.

The gram negative bacterium *E. coli* is widely used as a host for heterologous gene expression. However, large amounts of heterologous protein tend to accumulate inside the cell. Subsequent purification of the desired protein from the bulk of E.coli intracellular proteins can sometimes be difficult.

In contrast to *E.coli*, bacteria from the genus *Bacillus* are very suitable as heterologous hosts because of their capability to secrete proteins into the culture medium. Other bacteria suitable as hosts are those from the genera Streptomyces and Pseudomonas.

Depending on the nature of the polynucleotide encoding the polypeptide of the present invention, and/or the desirability for further processing of the expressed protein, eukaryotic hosts such as yeasts or other fungi may be preferred. In general, yeast cells are preferred over fungal cells because they are easier to manipulate. However, some proteins are either poorly secreted from the yeast cell, or in some cases are not processed properly (e.g. hyperglycosylation in yeast). In these instances, a different fungal host organism should be selected.

Examples of suitable expression hosts within the scope of the present invention are fungi such as Aspergillus species (such as those described in EP-A-0184438 and EP-A-0284603) and Trichoderma species; bacteria such as Bacillus species (such as those described in EP-A-0134048 and EP-A-0253455), Streptomyces species and Pseudomonas species; and yeasts such as Kluyveromyces species (such as those described in EP-A-0096430 and EP-A-0301670) and Saccharomyces species. By way of example, typical expression hosts may be selected from Aspergillus niger, Aspergillus niger var. tubigenis, Aspergillus niger var. awamori, Aspergillus aculeatis, Aspergillus nidulans, Aspergillus orvzae, Trichoderma reesei, Bacillus subtilis, Bacillus licheniformis, Bacillus amyloliquefaciens, Kluyveromyces lactis and Saccharomyces cerevisiae.

Polypeptides that are extensively modified may require correct processing to complete their function. In those instances, mammalian cell expression systems (such as HEK-293, CHO, HeLA) are required, and the polypeptides are expressed either intracellularly, on the cell membranes, or secreted in the culture media if preceded by an appropriate leader sequence.

The use of suitable host cells - such as yeast, fungal, plant and mammalian host cells - may provide for post-translational modifications (e.g. myristoylation, glycosylation, truncation, lapidation and tyrosine, serine or threonine phosphorylation) as may be needed to confer optimal biological activity on recombinant expression products of the present invention.

ORGANISM

The term "organism" in relation to the present invention includes any organism that could comprise the target according to the present invention and/or products obtained therefrom. Examples of organisms may include a fungus, yeast or a plant.

The term "transgenic organism" in relation to the present invention includes any organism that comprises the target according to the present invention and/or products obtained.

TRANSFORMATION OF HOST CELLS/HOST ORGANISMS

As indicated earlier, the host organism can be a prokaryotic or a eukaryotic organism. Examples of suitable prokaryotic hosts include *E. coli* and *Bacillus subtilis*. Teachings on the transformation of prokaryotic hosts is well documented in the art, for example see Sambrook *et al* (Molecular Cloning: A Laboratory Manual, 2nd edition, 1989, Cold Spring Harbor Laboratory Press) and Ausubel et al., Current Protocols in Molecular Biology (1995), John Wiley & Sons, Inc.

If a prokaryotic host is used then the nucleotide sequence may need to be suitably modified before transformation - such as by removal of introns.

In another embodiment the transgenic organism can be a yeast. In this regard, yeast have also been widely used as a vehicle for heterologous gene expression. The

species *Saccharomyces cerevisiae* has a long history of industrial use, including its use for heterologous gene expression. Expression of heterologous genes in *Saccharomyces cerevisiae* has been reviewed by Goodey et al (1987, Yeast Biotechnology, D R Berry et al, eds, pp 401-429, Allen and Unwin, London) and by King et al (1989, Molecular and Cell Biology of Yeasts, E F Walton and G T Yarronton, eds, pp 107-133, Blackie, Glasgow).

For several reasons *Saccharomyces cerevisiae* is well suited for heterologous gene expression. First, it is non-pathogenic to humans and it is incapable of producing certain endotoxins. Second, it has a long history of safe use following centuries of commercial exploitation for various purposes. This has led to wide public acceptability. Third, the extensive commercial use and research devoted to the organism has resulted in a wealth of knowledge about the genetics and physiology as well as large-scale fermentation characteristics of *Saccharomyces cerevisiae*.

A review of the principles of heterologous gene expression in *Saccharomyces cerevisiae* and secretion of gene products is given by E Hinchcliffe E Kenny (1993, "Yeast as a vehicle for the expression of heterologous genes", Yeasts, Vol 5, Anthony H Rose and J Stuart Harrison, eds, 2nd edition, Academic Press Ltd.).

Several types of yeast vectors are available, including integrative vectors, which require recombination with the host genome for their maintenance, and autonomously replicating plasmid vectors.

In order to prepare the transgenic Saccharomyces, expression constructs are prepared by inserting the nucleotide sequence of the present invention into a construct designed for expression in yeast. Several types of constructs used for heterologous expression have been developed. The constructs contain a promoter active in yeast fused to the nucleotide sequence of the present invention, usually a promoter of yeast origin, such as the GAL1 promoter, is used. Usually a signal sequence of yeast origin, such as the sequence encoding the SUC2 signal peptide, is used. A terminator active in yeast ends the expression system.

For the transformation of yeast several transformation protocols have been developed. For example, a transgenic Saccharomyces according to the present invention can be prepared by following the teachings of Hinnen et al (1978, Proceedings of the National Academy of Sciences of the USA 75, 1929); Beggs, J D

(1978, Nature, London, 275, 104); and Ito, H et al (1983, J Bacteriology 153, 163-168).

The transformed yeast cells are selected using various selective markers. Among the markers used for transformation are a number of auxotrophic markers such as LEU2, HIS4 and TRP1, and dominant antibiotic resistance markers such as aminoglycoside antibiotic markers, eg G418.

Another host organism is a plant. The basic principle in the construction of genetically modified plants is to insert genetic information in the plant genome so as to obtain a stable maintenance of the inserted genetic material. Several techniques exist for inserting the genetic information, the two main principles being direct introduction of the genetic information and introduction of the genetic information by use of a vector system. A review of the general techniques may be found in articles by Potrykus (Annu Rev Plant Physiol Plant Mol Biol [1991] 42:205-225) and Christou (Agro-Food-Industry Hi-Tech March/April 1994 17-27). Further teachings on plant transformation may be found in EP-A-0449375.

Further hosts suitable for the nucleotide sequence of the present invention include higher eukaryotic cells, such as insect cells or vertebrate cells, particularly mammalian cells, including human cells, or nucleated cells from other multicellular organisms. In recent years propagation of vertebrate cells in culture (tissue culture) has become a routine procedure. Examples of useful mammalian host cell lines are epithelial or fibroblastic cell lines such as Chinese hamster ovary (CHO) cells, NIH 3T3 cells, HeLa cells or 293T cells.

The nucleotide sequence of the present invention may be stably incorporated into host cells or may be transiently expressed using methods known in the art. By way of example, stably transfected mammalian cells may be prepared by transfecting cells with an expression vector having a selectable marker gene, and growing the transfected cells under conditions selective for cells expressing the marker gene. To prepare transient transfectants, mammalian cells are transfected with a reporter gene to monitor transfection efficiency.

To produce such stably or transiently transfected cells, the cells should be transfected with a sufficient amount of the nucleotide sequence of the present invention. The precise amounts of the nucleotide sequence of the present invention may be empirically determined and optimised for a particular cell and assay.

Thus, the present invention also provides a method of transforming a host cell with a nucleotide sequence that is to be the target or is to express the target. Host cells transformed with the nucleotide sequence may be cultured under conditions suitable for the expression of the encoded protein. The protein produced by a recombinant cell may be displayed on the surface of the cell. If desired, and as will be understood by those of skill in the art, expression vectors containing coding sequences can be designed with signal sequences which direct secretion of the coding sequences through a particular prokaryotic or eukaryotic cell membrane. Other recombinant constructions may join the coding sequence to nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins (Kroll DJ et al (1993) DNA Cell Biol 12:441-53).

AGENT

The agent may be an organic compound or other chemical. The agent can be an amino acid sequence or a chemical derivative thereof, or a combination thereof. The agent may even be a nucleotide sequence - which may be a sense sequence or an anti-sense sequence. The agent may even be an antibody.

Typically, the agents will be organic compounds. Typically the organic compounds will comprise two or more hydrocarbyl groups. Here, the term "hydrocarbyl group" means a group comprising at least C and H and may optionally comprise one or more other suitable substituents. Examples of such substituents may include halo-, alkoxy-, nitro-, an alkyl group, a cyclic group etc. In addition to the possibility of the substituents being a cyclic group, a combination of substituents may form a cyclic group. If the hydrocarbyl group comprises more than one C then those carbons need not necessarily be linked to each other. For example, at least two of the carbons may be linked *via* a suitable element or group. Thus, the hydrocarbyl group may contain hetero atoms. Suitable hetero atoms will be apparent to those skilled in the art and include, for instance, sulphur, nitrogen and oxygen. For some applications, preferably the agent comprises at least one cyclic group,. The cyclic group may be a polycyclic group, such as a non-fused polycyclic group. For some applications, the agent comprises at least the one of said cyclic groups linked to another hydrocarbyl group.

Examples of agents may be the compounds disclosed in GB patent application No. 9922702.7 (filed 25 September 1999). The teachings of that patent application are annexed hereto (after the Sequence Listings). For ease of reference, the pages of this application (which are numbered 1 etc.) bear the Header "P60191". For the avoidance of doubt, the teachings of that application are also a part of this application.

THERAPY

The agents identified by the assay method of the present invention may be used as therapeutic agents – i.e. in therapy applications.

The term "therapy" includes curative effects, alleviation effects, and prophylactic effects.

The therapy may be on humans or animals.

If any agent(s) adversely modulate the CCR5/gp120 interaction, then those agent(s) may be useful in the treatment of anti-inflammatory diseases and conditions and in the treatment and prevention of HIV-1 and genetically related retroviral infections.

HIV

In one aspect of the present invention, the assay method is used to identify agents that could be used to treat or prevent the spread or onset of a retroviral infection, especially human immunodeficiency virus (HIV) infection – i.e. treating or preventing HIV infection – and/or as treating or preventing AIDS.

The expressions "treating or preventing AIDS", and "preventing or treating infection by HIV" as used herein are intended to mean the treatment of a wide range of states of HIV infection: AIDS, ARC (AIDS related complex), both symptomatic and asymptomatic, and actual or potential exposure to HIV. The quoted expressions are not intended, however, to be limited to the recited treatments, but rather are contemplated to include all beneficial uses relating to conditions attributable to an AIDS causative agent. For example, the identified agents may be useful in treating infection by HIV after suspected past exposure to HIV by, e.g., blood transfusion, organ transplant, exchange of body fluids, sexual intercourse, bites, needle stick, or exposure to patient blood. In addition, an identified agent may be used for the prevention of infection by HIV and the prevention of AIDS, such as in pre-or post-coital prophylaxis or in the prevention of maternal transmission of the HIV virus to a fetus or a child, whether at the time of birth, during the period of nursing, or in any other manner.

With respect to the spread of HIV infection, it has been shown that for entry into target cells, human immunodeficiency viruses require a chemokine receptor, CCR5 and CXCR-4 among others, as well as the virus's primary receptor CD4. The principal cofactor for entry mediated by the envelope glycoproteins of primary macrophage-tropic strains of HIV-1 is CCR5. Deng, et al., Nature, 381, 661-666 (1996) further discuss aspects of CCR5 mediated HIV entry.

In short, HIV attaches to the CD4 molecule on cells through a region of its envelope protein, gp120, and gp120 is part of a multi-subunit complex, most likely a trimer of gp160, i.e., gp120 + gp41. It is believed that the CD4 binding site on the gp120 of HIV interacts with the CD4 molecule on the cell surface, triggering conformational changes across the trimer, which allow it to bind to another cell-surface receptor, such as CCR5. This in turn enables gp41 to induce fusion with the cell membrane, and entry of the viral core into the cell. In addition, macrophage-tropic HIV and SIV envelope proteins have been shown to induce a signal through CCR5 on CD4+ cells, which may enhance the replication of the virus. See Weissman, et al., Nature, 389, 981-985 (1997) for a description of this phenomenon. Further, it has been shown that a complex of gp120 and soluble CD4 interacts specifically with CCR5 and inhibits the binding of the natural CCR5 ligands, as described in Wu, et al., Nature, 384, 179-183 (1996); and Trkola, et al., Nature, 384, 184-187 (1996). It has further been demonstrated that β-chemokines and related molecules, e.g., (AOP)-RANTES, prevent HIV fusion to the cell membrane and subsequent infection, both in vitro, as described in Dragic, et al., Nature, 381, 667-673 (1996), and in animal models. Finally, absence of CCR5 appears to confer protection from HIV-1 infection, as described in Nature, 382, 668-669 (1996). In particular, an inherited frame-shifting mutation in the CCR5 gene has been shown to abolish functional expression of the gene in vitro, and individuals homozygous for the mutation are apparently not susceptible to HIV infection, while at the same time they do not seem to be immuno-compromised by this variant. Furthermore, those heterozygote individuals that have been infected by HIV progress more slowly to full-blown clinical AIDS. In addition to validating the role of CCR5 in the infectious cycle of HIV, the above observations suggest that CCR5 is dispensable in the adult organism.

Although most HIV-1 isolates studied to date utilize CCR5 or CXCR-4, at least nine other chemokine receptors, or structurally related molecules, have also been described as supporting HIV-1 env-mediated membrane fusion or viral entry in vitro. These include CCR2b, CCR3, BOB/GPR15, Bonzo/STRL33/TYMSTR, GPR1,

CCR8, US28, V28/CX3CR1, LTB-4, and APJ. There is good evidence that CCR3 can be used efficiently by a significant fraction of HIV-1 isolates in vitro, provided that this protein is over-expressed in transfected cells. Nevertheless, consistent evidence indicates that anti-HIV drugs targeted to chemokine receptors may not be compromised by this variability. Indeed, the chemokines RANTES, MIP-1α, SDF-1 have been shown to suppress replication of primary HIV isolates. A derivative of RANTES, (AOP)-RANTES, is a sub-nanomolar antagonist of CCR5 function in monocytes. Monoclonal antibodies to CCR5 have been reported to block infection of cells by HIV in vitro. A small molecule antagonist of CXCR4, identified as AMD3100, has been reported to inhibit infection of susceptible cultures by CXCR4 dependent primary and lab-adapted HIV viruses while another small molecule called TAK 779 blocks entry of CCR5-tropic strains (Baba, et al. PNAS, 96 (10), 5698-5703 (1999); In addition, the majority of primary strains from early and late disease stages utilize CCR5 exclusively or in addition to other chemokine receptors, indicating that CCR5 dependent infection may play an essential role in the initiation and maintenance of productive HIV infection in a host. Accordingly, an agent which blocks or adversely modulates CCR5 in patients including mammals, and especially humans who possess normal chemokine receptors, can reasonably be expected to prevent infection in healthy individuals and slow or halt viral progression in infected patients.

ADDITIONAL BIOLOGICAL STUDIES

If desired, the agents identified by the assay method of the present invention can be further investigated using other assay systems.

By way of example, referance may be made to the CCR5 binding assay procedures disclosed in Combadiere et al., J. Leukoc. Biol. 60, 147-52 (1996); and/or intracellular calcium mobilisation assays as described by the same authors. Cell lines expressing the receptor of interest include those naturally expressing the receptor, such as PM-1, or IL-2 stimulated peripheral blood lymphocytes (PBL), or a cell engineered to express a recombinant receptor, such as CHO, 300.19, L1.2 or HEK-293. In particular, the agents may be further investigated to see if they can prevent the binding of further known chemokine ligands to CCR5 In addition, the agents may be studied further to see if they prevent intracellular calcium mobilization in response to endogenous agonists, which is consistent with their functioning as CCR5 antagonists.

Further examples of other assays include those the HIV microculture assays described in Dimitrov et al., J. Clin. Microbiol. 28, 734-737 (1990)), and the pseudotyped HIV reporter assay described in Connor et al., Virology 206 (2) 935-44 (1995). In particular, the agents can be investigated to see if they inhibit p24 production following replication of laboratory-adapted and primary HIV strains in primary blood lymphocytes (PBLs) and clonal cell-lines known to support replication of both CCR5 and CXCR-4 tropic viruses, e.g., PM-1 and MOLT4-clone 8. Furthermore, the agents can be investigated to see if they inhibit entry of chimeric HIV reporter viruses pseudotyped with envelope from a CCR5 dependent strain (ADA). Furthermore, the agents can be investigated to see if they inhibit infection of primary cells by HIV isolated from infected patient blood.

PHARMACEUTICAL COMPOSITIONS

The present invention also provides a pharmaceutical composition comprising administering a therapeutically effective amount of the agent of the present invention and a pharmaceutically acceptable carrier, diluent or excipients (including combinations thereof).

The pharmaceutical compositions may be for human or animal usage in human and veterinary medicine and will typically comprise any one or more of a pharmaceutically acceptable diluent, carrier, or excipient. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art, and are described, for example, in Remington's Pharmaceutical Sciences, Mack Publishing Co. (A. R. Gennaro edit. 1985). The choice of pharmaceutical carrier, excipient or diluent can be selected with regard to the intended route of administration and standard pharmaceutical practice. The pharmaceutical compositions may comprise as - or in addition to - the carrier, excipient or diluent any suitable binder(s), lubricant(s), suspending agent(s), coating agent(s), solubilising agent(s).

Preservatives, stabilizers, dyes and even flavoring agents may be provided in the pharmaceutical composition. Examples of preservatives include sodium benzoate, sorbic acid and esters of p-hydroxybenzoic acid. Antioxidants and suspending agents may be also used.

There may be different composition/formulation requirements dependent on the different delivery systems. By way of example, the pharmaceutical composition of

the present invention may be formulated to be delivered using a mini-pump or by a mucosal route, for example, as a nasal spray or aerosol for inhalation or ingestable solution, or parenterally in which the composition is formulated by an injectable form, for delivery, by, for example, an intravenous, intramuscular or subcutaneous route. Alternatively, the formulation may be designed to be delivered by both routes.

Where the agent is to be delivered mucosally through the gastrointestinal mucosa, it should be able to remain stable during transit though the gastrointestinal tract; for example, it should be resistant to proteolytic degradation, stable at acid pH and resistant to the detergent effects of bile.

Where appropriate, the pharmaceutical compositions can be administered by inhalation, in the form of a suppository or pessary, topically in the form of a lotion, solution, cream, ointment or dusting powder, by use of a skin patch, orally in the form of tablets containing excipients such as starch or lactose, or in capsules or ovules either alone or in admixture with excipients, or in the form of elixirs, solutions or suspensions containing flavouring or colouring agents, or they can be injected parenterally, for example intravenously, intramuscularly or subcutaneously. For parenteral administration, the compositions may be best used in the form of a sterile aqueous solution which may contain other substances, for example enough salts or monosaccharides to make the solution isotonic with blood. For buccal or sublingual administration the compositions may be administered in the form of tablets or lozenges which can be formulated in a conventional manner.

PHARMACEUTICAL COMBINATIONS

The agent of the present invention may be administered with one or more other pharmaceutically active substances. By way of example, the present invention covers the simultaneous, or sequential treatments with an agent according to the present invention and one or more inhibitors of HIV protease and/or inhibitors of HIV reverse transcriptase, preferably selected from the class of non-nucleoside reverse transcriptase inhibitors (NNRTI), including but not limited to nevirapine, delavirdine, and efavirenz; from among the nucleoside/nucleotide inhibitors, including but not limited to zidovudine, didanosine, zalcitabine, stavudine, lamivudine, abacavir, and adefovir dipivoxil; and from among the protease inhibitors, including but not limited to indinavir, ritonavir, saquinavir, nelfinavir, and amprenavir. Other agents useful in the above-described preferred embodiment combinations of the present invention

include current and to-be-discovered investigational drugs from any of the above classes of inhibitors, including but not limited to FTC, PMPA, fozivudine tidoxil, talviraline, S-1153, MKC-442, MSC-204, MSH-372, DMP450, PNU-140690, ABT-378, and KNI-764.

There is also included within the scope of the preferred embodiments of the present invention, combinations of an agent according to the present invention together with a supplementary therapeutic agent used for the purpose of auxiliary treatment, wherein said supplementary therapeutic agent comprises one or more members independently selected from the group consisting of proliferation inhibitors, e.g., hydroxyurea; immunomodulators, e.g., sargramostim, and various forms of interferon or interferon derivatives; fusion inhibitors, e.g., AMD3100, T-20, PRO-542, AD-349, BB-10010 and other chemokine receptor agonists/antagonists; integrase inhibitors, e.g., AR177; RNaseH inhibitors; inhibitors of viral transcription and RNA replication; and other agents that inhibit viral infection or improve the condition or outcome of HIV-infected individuals through different mechanisms.

For some applications, preferred methods of treatment of the present invention for the prevention of HIV infection, or treatment of aviremic and asymptomatic subjects potentially or effectively infected with HIV, include but are not limited to administration of a member independently selected from the group consisting of: (i) an agent according to the present invention; (ii) one NNRTI in addition to a compound of (i); (iii) two NRTI in addition to a compound of (i); (iv) one NRTI in addition to the combination of (ii); and (v) a compound selected from the class of protease inhibitors used in place of an NRTI in combinations (iii) and (iv).

For some applications, preferred methods of treatment of the present invention for the therapy of HIV-infected individuals with detectable viremia or abnormally low CD4 counts further include as a member to be selected: (vi) treatment according to (i) above in addition to the standard recommended initial regimens for the therapy of established HIV infections, e.g., as described in Bartlett, J. G., "1998 Medical management of HIV infection", Johns Hopkins University publishers, ISBN 0-9244-2809-0. Such standard regimens include but are not limited to an agent from the class of protease inhibitors in combination with two NRTIs; and (vii) a standard recommended initial regimens for the therapy of established HIV infections, e.g., as described in Bartlett, J. G., "1998 Medical management of HIV infection", Johns Hopkins University publishers, ISBN 0-9244-2809-0), where either the protease

inhibitor component, or one or both of the NRTIs is/are replaced by an agent according to the present invention.

For some applications, preferred methods of treatment of the present invention for the therapy of HIV-infected individuals that have failed antiviral therapy further include as a member to be selected: (viii) treatment according to (i) above, in addition to the standard recommended regimens for the therapy of such patients, e.g., as described in Bartlett, J. G., "1998 Medical management of HIV infection", Johns Hopkins University publishers, ISBN 0-9244-2809-0); and (ix) a standard recommended initial regimens for the therapy of patients who have failed antiretroviral therapy, e.g., as described in Bartlett, J. G., "1998 Medical management of HIV infection", Johns Hopkins University publishers, ISBN 0-9244-2809-0), where either one of the protease inhibitor components, or one or both of the NRTIs is/are replaced by an agent according to the present invention.

In the above-described combinations of the present invention, the agent of the present invention and other therapeutic active agents may be administered in terms of dosage forms either separately or in conjunction with each other, and in terms of their time of administration, either serially or simultaneously. Thus, the administration of one component agent may be prior to, concurrent with, or subsequent to the administration of the other component agent(s).

ADMINISTRATION

Typically, a physician will determine the actual dosage which will be most suitable for an individual subject and it will vary with the age, weight and response of the particular patient. The dosages below are exemplary of the average case. There can, of course, be individual instances where higher or lower dosage ranges are merited.

The compositions of the present invention may be administered by direct injection. The composition may be formulated for parenteral, mucosal, intramuscular, intravenous, subcutaneous, intraocular or transdermal administration. Depending upon the nned, the agent may be administered at a dose of from 0.01 to 30 mg/kg body weight, such as from 0.1 to 10 mg/kg, more preferably from 0.1 to 1 mg/kg body weight.

By way of further example, the agents of the present invention may be administered in accordance with a regimen of 1 to 4 times per day, preferably once or twice per day. The specific dose level and frequency of dosage for any particular patient may be varied and will depend upon a variety of factors including the activity of the specific compound employed, the metabolic stability and length of action of that compound, the age, body weight, general health, sex, diet, mode and time of administration, rate of excretion, drug combination, the severity of the particular condition, and the host undergoing therapy. In particular, however, the treatment of retroviral infections, and more particularly HIV, may be guided by genotyping and phenotyping the virus in the course of or prior to the initiation of administration of the therapeutic agent. In this way, it is possible to optimise dosing regimens and efficacy when administering an agent according to the present invention for the prevention or treatment of infection by a retrovirus, in particular, the human immunodeficiency virus (HIV).

The term "administered" includes delivery by viral or non-viral techniques. Viral delivery mechanisms include but are not limited to adenoviral vectors, adeno-associated viral (AAV) vectos, herpes viral vectors, retroviral vectors, lentiviral vectors, and baculoviral vectors. Non-viral delivery mechanisms include lipid mediated transfection, liposomes, immunoliposomes, lipofectin, cationic facial amphiphiles (CFAs) and combinations thereof. The routes for such delivery mechanisms include but are not limited to mucosal, nasal, oral, parenteral, gastrointestinal, topical, or sublingual routes.

The term "administered" includes but is not limited to delivery by a mucosal route, for example, as a nasal spray or aerosol for inhalation or as an ingestable solution; a parenteral route where delivery is by an injectable form, such as, for example, an intravenous, intramuscular or subcutaneous route.

For some applications, preferably the agent is administered orally.

GENERAL RECOMBINANT DNA METHODOLOGY TECHNIQUES

Although in general the techniques mentioned herein are well known in the art, reference may be made in particular to Sambrook et al., Molecular Cloning, A Laboratory Manual (1989) and Ausubel et al., Short Protocols in Molecular Biology (1999) 4th Ed, John Wiley & Sons, Inc. PCR is described in US-A-4683195, US-A-

4800195 and US-A-4965188.

SUMMARY

Thus, in summary, the present invention provides an assay method for determining whether an agent is capable of modulating the interaction of CCR5 with gp120. The assay method comprises incubating the agent with CCR5 and gp120 to form a first reaction mixture; and determining whether said agent modulates the interaction of CCR5 with gp120. In the method, gp120 is associated with CD4. In particular, in the assay method the interaction of CCR5 with gp120 is a low affinity binding.

The present invention also relates to agents identified using said method, as well as pharmaceutical compositions comprising same, as well as methods of therapy using same.

In a preferred embodiment the present invention provides an assay method for determining whether an agent is capable of modulating the interaction of CCR5 with gp120; the method comprising: incubating the agent with CCR5 and gp120 to form a first reaction mixture; and determining whether said agent modulates the interaction of CCR5 with gp120; wherein said gp120 is associated with CD4; wherein said interaction is a low affinity binding; and wherein said method includes the step of adding a ligand to said first reaction mixture to form a second reaction mixture; wherein said ligand is capable of indicating whether said agent has modulated said interaction; and wherein said ligand has a detectable label.

In a highly preferred embodiment the present invention provides an assay method for determining whether an agent is capable of modulating the interaction of CCR5 with gp120; the method comprising: incubating the agent with CCR5 and gp120 to form a first reaction mixture; and determining whether said agent modulates the interaction of CCR5 with gp120; wherein said gp120 is associated with CD4; wherein said interaction is a low affinity binding; and wherein said method includes the step of adding a ligand to said first reaction mixture to form a second reaction mixture; wherein said ligand is capable of indicating whether said agent has modulated said interaction; wherein said ligand has a detectable label; and wherein said ligand comprises at least a first antibody, wherein said first antibody is capable of binding to gp120 and wherein said binding is high affinity binding; and wherein said ligand possibly comprises a second antibody, wherein said second antibody is capable of

binding to said first antibody; and wherein said detectable label is associated with said second antibody, or with first antibody if second antibody is not required.

EXAMPLES

The present invention will now be described, by way of example only.

In accordance with the present invention, TR-FIA (time resolved fluorescence immunoassay) is used to determine IC50 of inhibition of gp120 binding to the receptor CCR-5 on MIP34.10 in 96 well plates.

The principle of this assay is based on competitive inhibition of gp120 binding to the receptor CCR-5 on transformed HEK-293 MIP34.10 cells. Varying concentrations of compounds and chemokines are incubated with a constant amount of the gp120, prior to addition of an anti-gp120 antibody and a secondary Eu^{3+} labelled anti-IgG. The displacement of the gp120/CD4 /antibody/antibody complex is measured by time resolved fluorescence (TRF) in a DELFIA Counter and % inhibitions are calculated using XL software.

MATERIALS AND METHODS :

Cell Culture

MIP34.10 cells supplied by Cell Biology and continued passage within the laboratory.
PBS (Dulbecco's) without Ca2+ and Mg2+ - HyQ Reagents,HyClone (cat no : B-4004-L)
1x Cell Dissociation Solution non-enzymatic - Sigma (cat no : C-5914)

Growth medium

500ml 1x Dulbecco's Modified Eagle's Medium (DMEM) with 3.7g/l sodium bicarbonate without L-glutamine - HyQ Reagents, HyClone (cat no : B-7501-L)
50ml foetal calf serum (FCS) - PAA Laboratories, Austria (cat no : A15-041)
5ml 200mM L-Glutamine - Gibco BRL (cat no : 25030-024)
5ml Penicillin/Streptomycin (100U/ml Pen/10mg/ml Strep) - Sigma (cat no : P-7539)
6.5ml 50mg/ml Geneticin - Gibco BRL (cat no : 10131-019)

162cm2 Cell Culture Flask Tissue Culture Treated - Costar (cat no : 3151)
Incubator set @ 37(C, 5% CO2 humidified

Labelling reagents

Labelling Buffer :

50mM NaHCO3 pH 8.5 - Sigma (cat no : S6014)
0.9% NaCl - Sigma (cat no : S5150)

Elution Buffer :
50mM Tris-HCl pH 7.8 - Sigma (cat no : T2913)
0.9% NaCl - Sigma (cat no : S5150)
0.05% NaN3- Sigma (cat no : S8032)

Donkey anti-sheep IgG - Sigma (cat no: S2763)
Delfia Eu-labelling kit - EG&G Wallac (cat no : 1244-302)
PD-10 Sephadex G-25 pre-packed columns - Amersham Pharmacia (cat no : 17-0851-01)
UV detector and tubing
Collection tubes
Spectrophotometer

Assay Reagents

Dilution / Wash 1 / Assay Buffer :
500ml 1x Dulbecco's Modified Eagle's Medium (DMEM) with 3.7g/l sodium bicarbonate without L-glutamine - HyQ Reagents, HyClone (cat no : B-7501-L)
50ml foetal calf serum (FCS) - PAA Laboratories, Austria (cat no : A15-041)

Wash 2 Buffers :
1) Wash concentrate (x25) - EG&G Wallac (cat no : 1380-0865/R)
dilute 1:25 with double distilled water

Dulbecco's PBS - Gibco BRL (cat no : 14190-094)

Enhancement
Enhancement solution - EG&G Wallac (cat no : 1244-104)

sCD4 (human recombinant T-cell) - ImmunoDiagnostics Inc (cat no : 7001)
M-Tropic BaL sgp120 produced in-house
Sheep anti-HIV-1 gp120 antibody (anti C terminal) - Aalto Bio Reagents Ltd, Ireland (cat no : D7324)
Eu3+ labelled donkey anti-sheep IgG - produced in-house as below
MIP-1 a, b or RANTES - R & D Systems (cat no : 270-LD-010, 271-BM-010 and 278-RN-010)
DMSO tissue culture grade - Sigma (cat no : D-2650)
Biocoat Cell Environments Poly-D-Lysine 96-well black/clear plates - Becton Dickinson (cat no : 6640)
Haemocytometer Counting Chamber
Multichannel Pipettes - Labsystems Finnpipette (cat no : 4510-000/020/030/040/050)
Pipette Tips - Radleys ABT Aerosol Barrier Tips (cat no : ABT-20/100/2000/1000)
Reagent Reservoirs for multichannel pipettes - Costar (cat no : 4870)
DELFIA Fluorometer (cat no : 1234-001)

Procedure

Cell Culture

MIP34.10 cells supplied by Cell Biology are cultured in 162cm2 cell culture flasks to a confluency between 50-70% in the above growth medium @ 37(C for 2-3 days in the humidified incubator.

The cells are grown at low density because of their clumpy nature and a tendency to lose their receptor at full confluency.

For cell passage, 5ml of cell dissociation solution (non-enzymatic) is added to a 1x washed (PBS) 162cm2 flask of cells, the flask rapped on the side to dislodge the cells and split according to usage, usually 1:5 or 1:10 will give sufficient cells for use within 2 -4 days.

Each 162cm2 flask used for the TR-FIA assay is washed once in 20ml PBS and 5ml cell dissociation solution (non-enzymatic) added, the flask is again rapped on the side to dislodge the cells.

The cells are placed into a 50ml centrifuge tube and 10-20ml assay buffer1 (care hould be taken not to overdilute or the cells will need to be spun down and resuspended in a smaller volume) is added into the flask to wash any residual cells out and then combined in the centrifuge tube.

The cells are gently shaken and then counted in a haemocytometer (do appropriate dilutions to enable a count).

Dilute the cells to a density of 1x106 cells/ml and plate 100mM into every well of the poly-D-Lysine plates and incubate overnight @ 37(C in the humidified incubator.

Eu^{3+} Antibody Preparation

Add 500ml of labelling buffer to the anti-sheep IgG to solubilize and transfer to the Eu-labelling reagent vial, mix thoroughly and incubate overnight at 28(C

To purify, equilibrate a PD-10 column with 3x the void volume of elution buffer, add the reaction mix and rinse the labelling vial with a small volume of elution buffer and elute.

Monitor the eluate by UV absorbance at 280nm, collect 1-2ml fractions.

Pool the protein peak fractions and measure the Eu^{3+} concentration.

Eu^{3+} Content : serially dilute above with enhancement solution from kit (1E-04 - 1E-06) and compare to the fluorescence of 1nM Eu^{3+} standard supplied in the kit.

Protein Content : calculated from the measured absorbance at 280nm after subtracting the absorbance of the formed aromatic thiourea bonds.

Calculations

Eu^{3+} mM = (Eu counts x dilution factor) / (1000 x counts of Eu standard)

Protein mg/ml = (Abs (280) - 0.008 x Eu mM) / 1.34

Protein mM = (protein mg/ml x 1000000) / 160000

Yield = Eu mM / protein mM

Recovery = 100 x protein mg/ml x volume of fraction ml / protein added mg

Compound Preparation

Solubilize 1mg compound in DMSO to a final concentration of 10mM.

2) Mix well to solubilize compound, Vortex for 10 seconds or sonibath for 2-5 minutes if necessary. Make a record of compound solubility.

Prepare a dilution series in assay buffer as necessary down at least 6 dilutions (recommended starting concn of 1(M).

Chemokines, if used. MIP-1 a, MIP-1 b and RANTES are prepared in assay buffer alone. These are sticky proteins and care must be taken on preparing these. 10(g of lyophilised material is resuspended to give a final concentration of 10(M (sonibath for 2 minutes) and dilution series prepared from this from 200nM down 6 three-fold dilutions. SP3 special tips are used for all dilutions (ABT aerosol barrier - see assay reagents above).

Assay Protocol

Add an equal mix of 36nM sCD4 (100mg/ml solution diluted 1/40 in assay buffer) and 60nM gp120 (1/120 of 1mg/ml) and incubate on ice for 15 minutes.

2) Wash the overnight plates in 100(l wash 1 buffer/well once.

Add 20(l compound or control chemokine except for the background control which is replaced with 40(l assay buffer.

Add 20(l gp120 to each background control well and add 40(l of the sCD4/gp120 complex to each well with compound and reaction control except for the background control wells.

Each mixture contains :

Reaction control : 20(l assay buffer
40(l gp120/sCD4 complex

Compound reaction : 20(l compound dilution
40(l gp120/sCD4 complex

Standard reaction : 20(l MIP-1 a, MIP-1 b or RANTES dilution
40(l gp120/sCD4 complex

Background control : 40(l binding buffer
20(l gp120

Incubate the 96 well plates @37(C on a rocking platform for 60 minutes.

Empty the plate, blot and wash with 100(l/well wash 1 buffer once.

7) Add 100(l/well of a 1/50 dilution of the sheep anti-HIV-1 gp120 antibody to each well and incubate @RT for 90 minutes with rocking.

8) Empty the plate, blot and wash with 100(l/well wash 1 buffer once.

9) Add 100(l/well of a 1/1000 dilution of the Eu3+ labelled donkey anti-sheep IgG antibody to each well and incubate @RT for 15 minutes with rocking.

10) Empty the plate, blot and wash with 100(l/well wash 2 buffer once.

Empty the plate, blot and wash with 100(l/well PBS buffer 2x.

12) Empty the plate, blot and add 200(l/well enhancement solution and vortex for 2-3 minutes.

13) The plates are read in a DELFIA 1234 Fluorometer, plates should be read between 15 and 60 minutes.

Data Analysis

All calculations are performed in Microsoft Excel or Genesis (Lab Systems version 2.12).

1) Averages and standard deviations of the replicates are computed, along with CVs.

5) An IC50 value is generated by a four point logistic sigmoid curve fit using XL software.

COMPOUNDS TESTED

Compounds of the type described above were tested in accordance with the present invention and were found to be effective in accordance with the present invention – i.e. they can modulate the interaction of CCR5 with gp120. In addition, we found that activity in the described assay correlates well with potency against HIV in microculture assays referenced above.

All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and system of the present invention will be apparent to those skilled in the art without departing from the scope and spirit of the present invention. Although the present invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in biochemistry and biotechnology or related fields are intended to be within the scope of the following claims.

REFERENCES FOR CCR5 SECTION

Alkhatib, G.; Combadiere, C.; Broder, C. C.; Feng, Y.; Kennedy, P. E.; Murphy, P. M.; Berger, E. A. : CC CKR5: a RANTES, MIP-1-α, MIP-1-β receptor as a fusion cofactor for macrophage-tropic HIV-1. Science 272: 1955-1958, 1996.

Alvarez, V.; Lopez-Larrea, C.; Coto, E. : Mutational analysis of the CCR5 and CXCR4 genes (HIV-1 co-receptors) in resistance to HIV-1 infection and AIDS development among intravenous drug users. Hum. Genet. 102: 483-486, 1998.

Ansari-Lari, M. A.; Liu, X.-M.; Metzker, M. L.; Rut, A. R.; Gibbs, R. A. : The extent of genetic variation in the CCR5 gene. (Letter) Nature Genet. 16: 221-222, 1997.

Biti, R.; Ffrench, R.; Young, J.; Bennetts, B.; Stewart, G.; Liang, T. : HIV-1 infection in an individual homozygous for the CCR5 deletion allele. (Letter) Nature Med. 3: 252-253, 1997.

Browning, J.; Horner, J. W.; Pettoello-Mantovani, M.; Raker, C.; Yurasov, S.; DePinho, R. A.; Goldstein, H. : Mice transgenic for human CD4 and CCR5 are susceptible to HIV infection. Proc. Nat. Acad. Sci. 94: 14637-14641, 1997.

Carrington, M.; Dean, M.; Martin, M. P.; O'Brien, S. J. : Genetics of HIV-1 infection: chemokine receptor CCR5 polymorphism and its consequences. Hum. Molec. Genet. 8: 1939-1945, 1999.

Choe, H.; Farzan, M.; Sun, Y.; Sullivan, N.; Rollins, B.; Ponath, P. D.; Wu, L.; Mackay, C. R.; LaRosa, G.; Newman, W.; Gerard, N.; Gerard, C.; Sodroski, J. : The β-chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. Cell 85: 1135-1148, 1996.

Cohen, O. J.; Vaccarezza, M.; Lam, G. K.; Baird, B. F.; Wildt, K.; Murphy, P. M.; Zimmerman, P. A.; Nutman, T. B.; Fox, C. H.; Hoover, S.; Adelsberger, J.; Baseler, M.; and 14 others : Heterozygosity for a defective gene in CC chemokine receptor 5 is not the sole determinant for the immunologic and virologic phenotype of HIV-infected long-term nonprogressors. J. Clin. Invest. 100: 1581-1589, 1997.

Dean, M.; Carrington, M.; Winkler, C.; Huttley, G. A.; Smith, M. W.; Allikmets, R.; Goedert, J. J.; Buchbinder, S. P.; Vittinghoff, E.; Gomperts, E.; Donfield, S.; Vlahov, D.; Kaslow, R.; Saah, A.; Rinaldo, C.; Detels, R.; Hemophilia Growth and Development Study; Multicenter AIDS Cohort Study; Multicenter Hemophilia Cohort Study; San Francisco City Cohort; ALIVE Study; O'Brien, S. J. : Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CKR5 structural gene. Science 273: 1856-1861, 1996.

Deng, H.; Liu, R.; Ellmeier, W.; Choe, S.; Unutmaz, D.; Burkhart, M.; Di Marzio, P.; Marmon, S.; Sutton, R. E.; Hill, C. M.; Davis, C. B.; Peiper, S. C.; Schall, T. J.; Littman, D. R.; Landau, N. R. : Identification of a major co-receptor for primary isolates of HIV-1. Nature 381: 661-666, 1996.

Dragic, T.; Litwin, V.; Allaway, G. P.; Martin, S. R.; Huang, Y.; Nagashima, K. A.; Cayanan, C.; Maddon, P. J.; Koup, R. A.; Moore, J. P.; Paxton, W. A. : HIV-1 entry into CD4(+) cells is mediated by the chemokine receptor CC-CKR-5. Nature 381: 667-673, 1996.

Farzan, M.; Mirzabekov, T.; Kolchinsky, P.; Wyatt, R.; Cayabyab, M.; Gerard, N. P.; Gerard, C.; Sodroski, J.; Choe, H. : Tyrosine sulfation of the amino terminus of CCR5 facilitates HIV-1 entry. Cell 96: 667-676, 1999.

Huang, Y.; Paxton, W. A.; Wolinsky, S. M.; Neumann, A. U.; Zhang, L.; He, T.; Kang, S.; Ceradini, D.; Jin, Z.; Yazdanbakhsh, K.; Kunstman, K.; Erickson, D.; Dragon, E.; Landau, N. R.; Phair, J.; Ho, D. D.; Koup, R. A. : The role of a mutant CCR5 allele in HIV-1 transmission and disease progression. Nature Med. 2: 1240-1243, 1996.

Husain, S.; Goila, R.; Shahi, S.; Banerjea, A. C. : First report of a healthy Indian heterozygous for delta-32 mutant of HIV-1 co-receptor-CCR5 gene. Gene 207: 141-147, 1998.

Libert, F.; Cochaux, P.; Beckman, G.; Samson, M.; Aksenova, M.; Cao, A.; Czeizel, A.; Claustres, M.; de la Rua, C.; Ferrari, M.; Ferrec, C.; Glover, G.; Grinde, B.; Guran, S.; Kucinskas, V.; Lavinha, J.; Mercier, B.; Ogur, G.; Peltonen, L.; Rosatelli, C.; Schwartz, M.; Spitsyn, V.; Timar, L.; Beckman, L.; Parmentier, M.; Vassart, G. : The delta-ccr5 mutation conferring protection against HIV-1 in Caucasian populations has a single and recent origin in northeastern Europe. Hum. Molec. Genet. 7: 399-406, 1998.

Liu, R.; Paxton, W. A.; Choe, S.; Ceradini, D.; Martin, S. R.; Horuk, R.; MacDonald, M. E.; Stuhlmann, H.; Koup, R. A.; Landau, N. R. : Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. Cell 86: 367-377, 1996.

Martin, M. P.; Dean, M.; Smith, M. W.; Winkler, C.; Gerrard, B.; Michael, N. L.; Lee, B.; Doms, R. W.; Margolick, J.; Buchbinder, S.; Goedert, J. J.; O'Brien, T. R.; Hilgartner, M. W.; Vlahov, D.; O'Brien, S. J.; Carrington, M. : Genetic acceleration of AIDS progression by a promoter variant of CCR5. Science 282: 1907-1911, 1998.

Martinson, J. J.; Chapman, N. H.; Rees, D. C.; Liu, Y.-T.; Clegg, J. B. : Global distribution of the CCR5 gene 32-basepair deletion. Nature Genet. 16: 100-103, 1997.

McDermott, D. H.; Zimmerman, P. A.; Guignard, F.; Kleeberger, C. A.; Leitman, S. F.; Murphy, P. M. : CCR5 promoter polymorphism and HIV-1 disease progression: multicenter AIDS cohort study (MACS). Lancet 352: 866-870, 1998.

Quillent, C.; Oberlin, E.; Braun, J.; Rousset, D.; Gonzalez-Canali, G.; Metais, P.; Montagnier, L.; Virelizier, J.-L.; Arenzana-Seisdedos, F.; Beretta, A. : HIV-1-resistance phenotype conferred by combination of two separate inherited mutations of CCR5 gene. Lancet 351: 14-18, 1998.

Rottman, J. B.; Ganley, K. P.; Williams, K.; Wu, L.; Mackay, C. R.; Ringler, D. J. : Cellular localization of the chemokine receptor CCR5: correlation to cellular targets of HIV-1 infection. Am. J. Path. 151: 1341-1351, 1997.

Samson, M.; Labbe, O.; Mollereau, C.; Vassart, G.; Parmentier, M. : Molecular cloning and functional expression of a new human CC-chemokine receptor gene. Biochemistry 35: 3362-3367, 1996.

Samson, M.; Libert, F.; Doranz, B. J.; Rucker, J.; Liesnard, C.; Farber, C.-M.; Saragosti, S.; Lapoumeroulie, C.; Cogniaux, J.; Forceille, C.; Muyldermans, G.; Verhofstede, C.; Burtonboy, G.; Georges, M.; Imai, T.; Rana, S.; Yi, Y.; Smyth, R. J.; Collman, R. G.; Doms, R. W.; Vassart, G.; Parmentier, M. : Resistance to HIV-1 infection in Caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. Nature 382: 722-725, 1996.

Smith, M. W.; Dean, M.; Carrington, M.; Winkler, C.; Huttley, G. A.; Lomb, D. A.; Goedert, J. J.; O'Brien, T. R.; Jacobson, L. P.; Kaslow, R.; Buchbinder, S.; Vittinghoff, E.; Vlahov, D.; Hoots, K.; Hilgartner, M. W.; Hemophilia Growth and Development Study (HGDS); Multicenter AIDS Cohort Study (MACS); Multicenter Hemophilia Cohort Study (MHCS); San Francisco City Cohort (SFCC); ALIVE Study; O'Brien, S. J. : Contrasting genetic influence of CCR2 and CCR5 variants on HIV-1 infection and disease progression. Science 277: 959-965, 1997.

Stephens, J. C.; Reich, D. E.; Goldstein, D. B.; Shin, H. D.; Smith, M. W.; Carrington, M.; Winkler, C.; Huttley, G. A.; Allikmets, R.; Schriml, L.; Gerrard, B.; Malasky, M.; and 27 others : Dating the origin of the CCR5-del32 AIDS-resistance allele by the coalescence of haplotypes. Am. J. Hum. Genet. 62: 1507-1515, 1998.

Zagury, D.; Lachgar, A.; Chams, V.; Fall, L. S.; Bernard, J.; Zagury, J.-F.; Bizzini, B.; Gringeri, A.; Santagostino, E.; Rappaport, J.; Feldman, M.; O'Brien, S. J.; Burny, A.; Gallo, R. C. : C-C chemokines, pivotal in protection against HIV type 1 infection. Proc. Nat. Acad. Sci. 95: 3857-3861, 1998.

Zimmerman, P. A.; Buckler-White, A.; Alkhatib, G.; Spalding, T.; Kubofcik, J.; Combadiere, C.; Weissman, D.; Cohen, O.; Rubbert, A.; Lam, G.; Vaccarezza, M.; Kennedy, P. E.; Kumaraswami, V.; Giorgi, J. V.; Detels, R.; Hunter, J.; Chopek, M.; Berger, E. A.; Fauci, A. S.; Nutman, T. B.; Murphy, P. M. : Inherited resistance to HIV-1 conferred by an inactivating mutation in CC chemokine receptor 5: studies in populations with contrasting clinical phenotypes, defined racial background, and quantified risk. Molec. Med. 3: 23-26, 1997.

REFERENCES FOR CD4 SECTION

Amadori, A.; Zamarchi, R.; De Silvestro, G.; Forza, G.; Cavatton, G.; Danieli, G. A.; Clememti, M.; Chieco-Bianchi, L. : Genetic control of the CD4/CD8 T-cell ratio in humans. Nature Med. 1: 1279-1283, 1995.

Chakravarti, A. : The CD4/CD8 ratio: message in a bottle? Nature Med. 1: 1240-1241, 1995.

Clementi, M.; Forabosco, P.; Amadori, A.; Zamarchi, R.; De Silvestro, G.; Di Gianantonio, E.; Chieco-Bianchi, L.; Tenconi, R. : CD4 and CD8 T lymphocyte inheritance: evidence for major autosomal recessive genes. Hum. Genet. 105: 337-342, 1999.

Kraal, G.; Weissman, I. L.; Butcher, E. C. : Genetic control of T-cell subset representation in inbred mice. Immunogenetics. 18: 585-592, 1983.

CLAIMS

1. An assay method for determining whether an agent is capable of modulating the interaction of CCR5 with gp120;

the method comprising:

incubating the agent with CCR5 and gp120 to form a first reaction mixture; and

determining whether said agent modulates the interaction of CCR5 with gp120;

wherein said gp120 is associated with CD4; and

wherein said interaction is a low affinity binding.

2. A method according to claim 1 wherein said method includes the step of adding a ligand to said first reaction mixture to form a second reaction mixture; wherein said ligand is capable of indicating whether said agent has modulated said interaction.

3. A method according to claim 2 wherein said ligand has a detectable label.

4. A method according to claim 3 wherein said detectable label is a fluorescent atom or a fluorescent group.

5. A method according to claim 5 wherein said radioactive atom is Eu^{3+}.

6. A method according to any one of claims 2 to 5 wherein said ligand comprises at least a first antibody.

7. A method according to claim 6 wherein said first antibody is capable of binding to gp120; and wherein said binding is high affinity binding, preferably wherein said first antibody is associated with a detectable label.

8. A method according to claim 6 or claim 7 wherein said ligand comprises at least a second antibody.

9. A method according to claim 7 wherein said second antibody is capable of binding to said first antibody.

10. A method according to claim 9 wherein said second antibody is an anti-IgG antibody.

11. A method according to any one of claims 8 to 10 when dependent on claim 3 wherein said detectable label is associated with said second antibody.

12. An agent identified by the method according to any one of claims 1 to 11, wherein said agent is capable of modulating the interaction of CCR5 with gp120.

13. A process comprising the steps of:

(a) performing the assay according to any one of claims 1 to 11;

(b) identifying one or more agents that are capable of modulating the interaction of CCR5 with gp120; and

(c) preparing a quantity of those one or more identified agents.

14. A method of affecting the *in vivo* interaction of CCR5 with gp120 with an agent;

wherein the agent is capable of modulating the interaction of CCR5 with gp120 in an *in vitro* assay method;

wherein the *in vitro* assay method is the assay method defined in any one of claims 1 to 11.

15. Use of an agent in the preparation of a pharmaceutical composition for the treatment of a disease or condition associated with the interaction of CCR5 with gp120, wherein the agent is the agent of claim 12 and/or wherein the agent is capable of modulating the interaction of CCR5 with gp120 when assayed *in vitro* by the assay method according to any one of claims 1 to 11.

16. A method of treating a subject with an agent, wherein the agent is the agent of claim 12 and/or wherein the agent is capable of modulating the interaction of CCR5 with gp120 when assayed *in vitro* by the assay method according to any one of claims 1 to 11.

ABSTRACT

METHOD

An assay method for determining whether an agent is capable of modulating the interaction of CCR5 with gp120 is disclosed. The method comprises incubating the agent with CCR5 and gp120 to form a first reaction mixture; and determining whether said agent modulates the interaction of CCR5 with gp120; wherein said gp120 is associated with CD4. In particular, the interaction is a low affinity binding.

SEQUENCE LISTINGS

CCR5

ID T90117 standard; cDNA; 1477 BP.
DE cDNA for human CCR5.
KW Human Cys-Cys chemokine receptor 5; CCR5; human immunodeficiency virus; type 1; type 2; HIV-1; HIV-2; diagnosis; treatment; prevention; inflammatory disease; rheumatoid arthritis; glomerulonephritis; asthma; idiopathic pulmonary fibrosis; psoriasis; viral infection; cancer; atherosclerosis; autoimmune disorder; ss.
OS Homo sapiens.
FH Key Location/Qualifiers
FT CDS 240..1298

SQ Sequence 1477 BP; 374 A; 349 C; 320 G; 431 T; 3 other

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gp120

Gp120 Sequences, HIV-1 BaL **strain**

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Amino-acid

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CD4

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envelope protein; T-lymphocyte.
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FT   Misc-difference 370
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ANNEX NOW FOLLOWS

CCR5 MODULATORS

This invention relates to new chemical compounds. These compounds find particular but not exclusive use as pharmaceuticals, especially as CCR5 antagonists and agonists.

This invention also relates to formulations or dosage forms including these compounds, to use of these compounds in manufacture of pharmaceutical formulations or dosage forms and methods of treatment, especially treatment of anti-inflammatory diseases and conditions and in the treatment and prevention of HIV-1 and genetically related retroviral infections.

The compositions of matter of the present invention may be modulators of the activity of chemokine CCR5 receptors, particularly those which occur on the surfaces of certain cells within the human body. Modulators of CCR5 receptor activity may be useful in the treatment and prevention of various inflammatory diseases and conditions, and in the treatment and prevention of infection by HIV-1 and genetically related retroviruses.

The name "chemokine", is a contraction of "chemotactic cytokines". The chemokines comprise a large family of proteins which have in common important structural features and which have the ability to attract leukocytes. As leukocyte chemotactic factors, chemokines play an indispensable role in the attraction of leukocytes to various tissues of the body, a process which is essential for both inflammation and the body's response to infection. Because chemokines and their receptors are central to the pathophysiology of inflammatory and infectious diseases, agents which are active in modulating, for example agonising or antagonizing, the activity of chemokines and their receptors, are useful in therapeutic treatment.

The chemokine receptor CCR5 is of particular importance in the context of treating inflammatory and infectious diseases. CCR5 is a receptor for chemokines, especially for the macrophage inflammatory proteins (MIP) designated MIP-1α and MIP-1β, and for a protein which is regulated upon activation and is normal T-cell expressed and secreted (RANTES). The relationship between modulators, especially antagonists of CCR5 activity and therapeutic usefulness in treating inflammation and HIV infection, and the manner in which such a relationship may be demonstrated, is explained in more detail further below.

There is ongoing in the art a substantial investigation of different classes of modulators of chemokine receptor activity, especially that of the CCR5 chemokine receptor. A representative disclosure is Mills *et al.* WO 98/25617 relating to substituted aryl piperazines as modulators of chemokine receptor activity. However, the compositions described therein are

not the same as, nor suggestive of those of the present invention. Further disclosures are: WO 98/025605; WO 98/025604; WO 98/002151; WO 98/004554; and WO 97/024325.

The present invention relates to compounds which may be conveniently considered to have four independently variable regions, reading from the left-hand side to right-hand side of said compound: R_{egion} α, R_{egion} β, R_{egion} γ, and R_{egion}δ, of Formula (I):

$$[R_{egion}\ \alpha] - [R_{egion}\ \beta] - [R_{egion}\ \gamma] - [R_{egion}\ \delta] \qquad (I)$$

and pharmaceutically acceptable salts and prodrug derivatives thereof. The compounds of the present invention may be selective CCR5 receptor modulators and are non-peptidyl in structure.

The compounds as exemplified by Formula (I) may contain one or more stereogenic centers and the present invention includes the recited compounds in both their separated and their unseparated forms. The separated forms can be obtained by conventional means, *e.g.*, by asymmetric synthesis, by using high performance liquid chromatography employing a chiral stationary phase, or by chemical resolution *via* the formation of suitable salts or derivatives. It will be understood that the separate optically active forms of the compositions of the present invention, as well as racemic mixtures thereof, will usually vary with respect to their biological properties because of the chirality-dependent conformation of the active site of an enzyme, receptor, *etc.*

The description which follows provides details of the particular moieties which comprise each of said R_{egions}. In order to present said details in an orderly and space-saving fashion, each major group in each Region is set out with a single dash (" - "), and each successive subdivision within each said group is set out in turn with two, three, etc. dashes as required.

In this specification and claims a reference to a range or class of groups for example $(C_1\text{-}C_3)$alkyl is to be understood as an express disclosure and reference of each member of the range or class, including isomers.

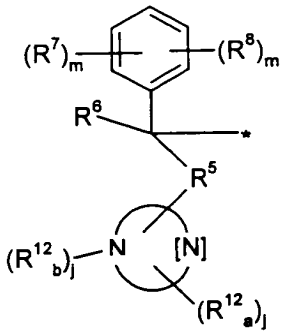
According to the present invention there is provided a compound of Formula (I);

$$[R_{egion}\ \alpha] - [R_{egion}\ \beta] - [R_{egion}\ \gamma] - [R_{egion}\ \delta] \qquad (I)$$

wherein **$[R_{egion}\ \alpha]$** is selected from the group consisting of:

-A. Aryl heterocyclyl substituent components comprising:

–1. hetero-phenylmethylene moieties of partial Formula (1.0.0):



(1.0.0)

—wherein: the symbol " * " indicates the point of attachment of the moiety of partial Formula (1.0.0) to R_{eglon} β, as hereinafter defined;

—R^5 is a member selected from the group consisting of a direct bond; -O-; -C(=O)-; -NR^4-; and -$S(=O)_p$-; where:

—R^4 is hydrogen or (C_1 -C_2)alkyl;

—R^6 is a member selected from the group consisting of hydrogen; (C_1 -C_2)alkyl; (C_1 -C_2)alkoxy; -CN; -OH; and -$C(=O)NH_2$;

—j is an integer selected from 0, 1, and 2;

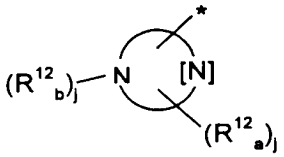
—m is an integer selected from 0, 1, and 2;

—R^7 and R^8 are each a member selected from the group consisting of -F; -Cl; -CO_2R^4; -OH; -CN; -$CONR^4_aR^4_b$; -$NR^4_aR^4_b$-; -$NR^4_aC(=O)R^4_b$; -$NR^4_aC(=O)OR^4_b$; -$NR^4_aS(=O)_pR^4_b$; -$S(=O)_pNR^4_aR^4_b$; (C_1 -C_4)alkyl, and (C_1 -C_4)alkoxy wherein said alkyl and alkoxy are each substituted with 0 to 3 substituents independently selected from F and Cl; (C_1 -C_2)alkoxycarbonyl; (C_1 -C_2)alkylcarbonyl; and (C_1 -C_2)alkylcarbonyloxy; where:

—p is an integer selected from 0, 1, and 2;

—R^4_a and R^4_b are each independently selected from hydrogen and (C_1 -C_2)alkyl;

—the moiety represented by partial Formula (1.0.1):



(1.0.1)

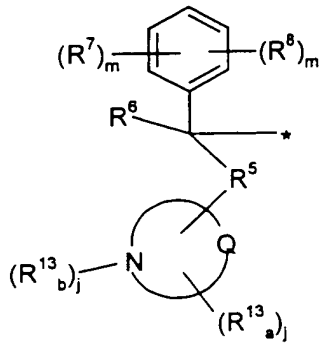
in partial Formula (1.0.0) represents a monocyclic heterocyclic group, or a bicyclic benzo-fused ring system containing said heterocyclic group wherein said heterocyclic group contains a total of 5- or 6- members of which one or two of said members is nitrogen, the presence of the optional second nitrogen atom being represented by: "[N]"; wherein said heterocyclic group or ring system are selected from the group consisting of pyrrolyl; pyrazolyl; imidazolyl; pyridinyl; pyrazinyl; pyrimidinyl; pyridazinyl; piperazinyl; indolyl; indazolinyl; benzimidazolyl; quinolinyl; *iso*-quinolinyl; and quinazolinyl; wherein:

—$R^{12}{}_a$ is a member selected from the group consisting of hydrogen; F; Cl; $-CO_2R^4$; oxo; -OH; CN; NH_2; $NH(C_1\text{-}C_2)$alkyl; $N(C_1\text{-}C_2)_2$dialkyl; $-CF_3$; $(C_1\text{-}C_4)$alkyl; $(C_2\text{-}C_4)$alkenyl; $(C_1\text{-}C_4)$alkoxy; $(C_3\text{-}C_7)$cycloalkyl; and phenyl; wherein said alkyl, alkenyl, alkoxy, cycloalkyl and phenyl are substituted with 0 to 2 substituents R^9 where:

——R^9 is a member independently selected from the group consisting of F; Cl; $-CO_2R^4$; -OH; cyano; $-CONR^4{}_aR^4{}_b$; $-NR^4{}_aR^4{}_b$-; $-NR^4{}_aC(=O)R^4{}_b$; $-NR^4{}_aC(=O)OR^4{}_b$; $-NR^4{}_aS(=O)_pR^4{}_b$; $-S(=O)_pNR^4{}_aR^4{}_b$; $(C_1\text{-}C_4)$alkyl including dimethyl, and $(C_1\text{-}C_4)$alkoxy wherein said alkyl and alkoxy are each independently substituted with 0 to 3 substituents independently selected from F and Cl; $(C_1\text{-}C_2)$alkoxycarbonyl; $(C_1\text{-}C_2)$alkylcarbonyl; and $(C_1\text{-}C_2)$alkylcarbonyloxy; and

—$R^{12}{}_b$ is absent or is a member selected from the group consisting of hydrogen; $(C_1\text{-}C_4)$alkyl; $(C_2\text{-}C_4)$alkenyl; $(C_1\text{-}C_2)$alkoxy; $(C_3\text{-}C_7)$cycloalkyl; and phenyl; wherein said alkyl, alkenyl, alkoxy, cycloalkyl and phenyl are substituted with 0 to 2 substituents R^9 wherein R^9 has the same meaning as above, except that it is selected independently selected therefrom; and

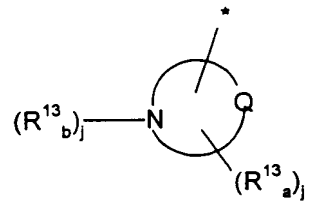
2. hetero-phenylmethylene moieties of partial Formula (1.1.0):



(1.1.0)

—wherein: the symbol " * "; R^5; R^6; R^7; R^8; j and m are as defined further above, except that all of the above-recited substituents are selected independently of their selection above;

—the moiety represented by partial Formula (1.1.1):



(1.1.1)

in partial Formula (1.1.0) represents:

—a. a monocyclic heterocyclic group containing a total of 5 or 6 members of which one said member is nitrogen and Q is selected from O and S where said S may optionally be in the sulfonate form, $-S(=O)_2$; wherein said heterocyclic group is selected from the group consisting of oxazolyl; oxazolidinyl; *iso*xazolyl; thiazolyl; thiazolidinyl; *iso*-thiazolyl; morpholinyl; and thiomorpholinyl; or

—b. a monocyclic heterocyclic group containing a total of 5- or 6- member s of which two said members are nitrogen and a third or fourth said member is independently selected from N, O, and S where said S may optionally be in the sulfonate form, $-S(=O)_2$; wherein said heterocyclic group is selected from the group consisting of triazolyl; triazinyl; tetrazolyl; oxadiazolyl; thiadiazolyl; and

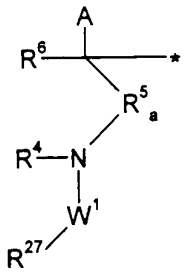
—$R^{13}{}_a$ is selected from the group consisting of hydrogen; F; Cl; $-CO_2R^4$; oxo; -OH; CN; NH_2; $NH(C_1\text{-}C_2)$alkyl; $N(C_1\text{-}C_2)_2$dialkyl; $-CF_3$; $(C_1\text{-}C_4)$alkyl; $(C_2\text{-}C_4)$alkenyl; $(C_1\text{-}C_2)$alkoxy; $(C_3\text{-}C_7)$cycloalkyl; and phenyl; wherein said alkyl, alkenyl, alkoxy, cycloalkyl and phenyl are substituted with 0 to 2 substituents R^{11} where:

——R^{11} is a member selected from the group consisting of F; Cl; $-CO_2R^4$; -OH; -CN; $-CONR^4{}_aR^4{}_b$; $-NR^4{}_aR^4{}_b$; $-NR^4{}_aC(=O)R^4{}_b$; $-NR^4{}_aC(=O)OR^4{}_b$; $-NR^4{}_aS(=O)_pR^4{}_b$; $-S(=O)_pNR^4{}_aR^4{}_b$; $(C_1\text{-}C_4)$alkyl including dimethyl, and $(C_1\text{-}C_4)$alkoxy wherein said alkyl and alkoxy are each independently substituted with 0 to 3 substituents independently selected from F and Cl; $(C_1\text{-}C_2)$alkoxycarbonyl; $(C_1\text{-}C_2)$alkylcarbonyl; and $(C_1\text{-}C_2)$alkylcarbonyloxy; and

—$R^{13}{}_b$ is a member selected from the group consisting of hydrogen; $(C_1\text{-}C_4)$alkyl; $(C_2\text{-}C_4)$alkenyl; $(C_1\text{-}C_2)$alkoxy; $(C_3\text{-}C_7)$cycloalkyl; $C(=O)(C_1\text{-}C_4)$alkyl; $S(=O)_2(C_1\text{-}C_4)$alkyl; and phenyl; wherein said alkyl, alkenyl, alkoxy, cycloalkyl and phenyl are substituted with 0 to 2 substituents R^{11} wherein R^{11} has the same meaning as in above, except that it is selected independently;

-B. a (substituted)-amido-aryl or -heterocyclyl moiety selected from the group consisting of

--1. alkyl-, alkenyl-, and alkynyl-substituted-amido-aryl moieties of partial Formula (2.0.0):

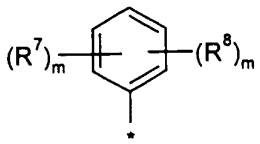


(2.0.0)

—wherein: the symbol " * "; R^4 and R^6; are as defined above, except that all of the above-recited substituents are selected independently of their selection above;

—A is a member selected from the group consisting of:

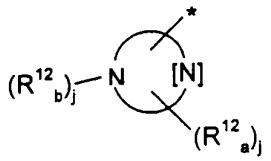
—1. the moiety of partial Formula (2.0.3)



(2.0.3)

——wherein: the symbol R^7; R^8 and m are as defined above, except that all of the above-recited substituents are selected independently of their selection above; and the symbol: " * " indicates the point of attachment of the moiety A to the, remaining portions of partial Formula (2.0.0);

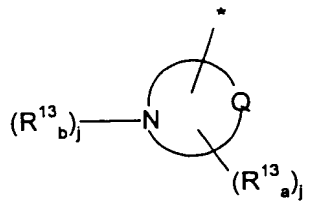
—2. the moiety of partial Formula (2.0.4)



(2.0.4)

which represents a monocyclic heterocyclic group, selected from the group consisting of pyrrolyl; pyrazolyl; imidazolyl; pyridinyl; pyrazinyl; pyrimidinyl; wherein: the symbol $R^{12}{}_a$ and $R^{12}{}_b$ are as defined above, except that all of the above-recited substituents are selected independently of their selection above; and the symbol: " * " indicates the point of attachment of the moiety A to the other, remaining portions of partial Formula (2.0.0);

—3. the moiety of partial Formula (2.0.5)



(2.0.5)

which represents

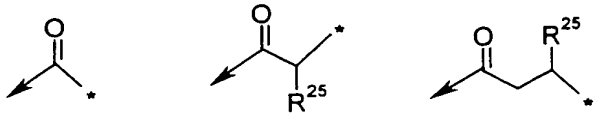
——a. a monocyclic heteroaromatic group containing a total of 5- members of which one said member is nitrogen and Q is selected from O and S where said S may optionally be in the sulfonate form, $-S(=O)_2$; selected from the group consisting of oxazolyl; *iso*xazolyl; thiazolyl; and *iso*-thiazolyl; or

——b. a monocyclic heterocyclic group containing a total of 5- or 6- members of which two said members are nitrogen and a third or fourth said member is independently selected from N, O, and S where said S may optionally be in the sulfonate form, $-S(=O)_2$; selected from the group consisting of triazolyl; triazinyl; tetrazolyl; oxadiazolyl; and thiadiazolyl; and

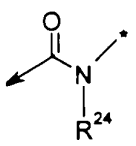
——wherein: the $R^{13}{}_a$, $R^{13}{}_b$ and j are as defined above, except that all of the above-recited substituents are selected independently of their selection above; and the symbol: " * " indicates the point of attachment of the moiety A to the other, remaining portions of partial Formula (2.0.2);

—$R^5{}_a$ is a member selected from the group consisting of a direct bond; -C(=O)-; and $-S(=O)_2-$;

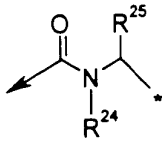
—W^1 is (1.) a direct bond; (2.) in the case where $R^5{}_a$ is -C(=O)- or $-S(=O)_2$, W^1 is a direct bond or $-(C_1\text{-}C_3)$alkylene- wherein any single carbon atom thereof is substituted by 0 to 2 substituents R^{23} where R^{23} is a member selected from the group consisting of -F; -Cl; $-CO_2R^4$; -OH; -CN; $(C_1\text{-}C_4)$alkoxy; $(C_3\text{-}C_7)$cycloalkyl; and phenyl; wherein said alkoxy, cycloalkyl, and phenyl are substituted with 0 to 2 substituents R^{11}, wherein said R^{11} is as defined above, except that all of the above-recited substituents are selected independently of their selection above; or (3.) is a member independently selected from the group consisting of the moieties of partial Formulas (2.0.6) through (2.0.16), inclusive:



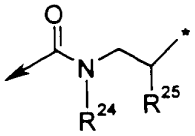
(2.0.6) (2.0.7) (2.0.8)



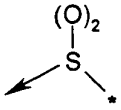
(2.0.9)



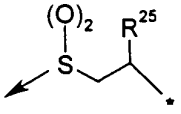
(2.0.10)



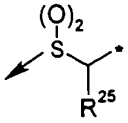
(2.0.11)



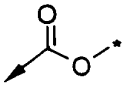
(2.0.12)



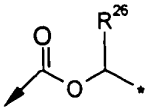
(2.0.13)



(2.0.14)



(2.0.15)



(2.0.16)

—wherein: the symbol: "→" indicates the point of attachment of the moiety W^1 to the nitrogen atom in partial Formula (2.0.0), and the symbol: " * " indicates the point of attachment of the moiety W^1 to the other, remaining portions of partial Formula (2.0.0); and R^4 is as defined further above, but selected on an independent basis;

——R^{24} is selected from the group consisting of hydrogen and $(C_1\text{-}C_4)$alkyl; and

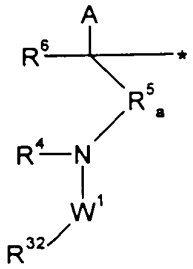
—R^{25} and R^{26} are each selected from the group consisting of -OH; $(C_1\text{-}C_2)$alkyl substituted by 0 to 3 substituents selected from F; and OH; and $(C_1\text{-}C_2)$alkoxy; and

—R^{27} is selected from the group consisting of $(C_1\text{-}C_6)$alkyl; $(C_2\text{-}C_6)$alkenyl; and $(C_2\text{-}C_6)$alkynyl; wherein said alkyl, alkenyl, and alkynyl groups comprising R^{27} are substituted with 0 to 3 substituents R^{28} where:

—R^{28} is selected from the group consisting of phenyl; F or Cl; oxo; hydroxy; $(C_1\text{-}C_2)$alkyl; $(C_1\text{-}C_3)$alkoxy; $-C(=O)OR^{29}$; $-C(=O)(C_1\text{-}C_4)$alkyl; $-S(=O)_2(C_1\text{-}C_4)$alkyl; $-C(=O)NR^{29}R^{30}$; $-NR^{29}R^{30}$; $-NR^{29}C(=O)R^{30}$; $-NR^{29}C(=O)OR^{30}$; $-NR^{29}S(=O)_pR^{30}$; and $-S(=O)_2NR^{29}R^{30}$, where:

——R^{29} and R^{30} are each a member independently selected from the group consisting of hydrogen and $(C_1\text{-}C_4)$alkyl substituted by 0 to 3 substituents selected from the group consisting of F and Cl;

–2. cycloalkyl-substituted-amido-aryl moieties of partial Formula (2.1.0):

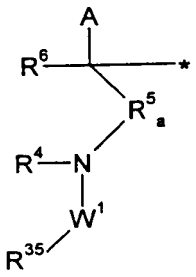


(2.1.0)

---wherein: A; W^1; the symbol " * "; R^4; $R^5{}_a$; and R^6 have the same meaning as set out above, except that all of the above-recited substituents are selected independently of their selection above; and

---R^{32} is a member selected from the group consisting of $-(CH_2)_n-(C_3\text{-}C_7)$cycloalkyl, where n is an integer selected from 0, 1, and 2; in the event n is 0, then the α-carbon atom of said $(C_3\text{-}C_7)$cycloalkyl is substituted by 0 or 1 $(C_1\text{-}C_4)$alkyl or phenyl, where said alkyl or phenyl are substituted by 0, 1, or 2 of CH_3, OCH_3, OH or NH_2; and in the event that n is 1 or 2, the resulting methylene or ethylene is substituted by 0 or 1 of F; NH_2; $N(CH_3)_2$; OH; OCH_3; $(C_1\text{-}C_4)$alkyl; or phenyl; where said alkyl and phenyl are substituted by 0, 1, or 2 of CH_3, OCH_3, OH, and NH_2; and further wherein said $(C_3\text{-}C_7)$cycloalkyl is substituted by 0 to 3 substituents R^{28} where R^{28} is as defined further above, but selected independently

--3. aryl and heterocyclic-substituted-amido-aryl moieties of partial Formula (2.2.0):



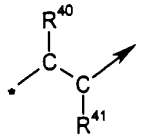
(2.2.0)

---wherein: A; W^1; the symbol: " * "; R^4; $R^5{}_a$; and R^6 have the same meaning as set out above, except that all of the above-recited substituents are selected independently of their selection above; and

---R^{35} is selected from the group consisting of phenyl; furyl; tetrahydrofuranyl; tetrahydropyranyl; oxetanyl; thienyl; pyrrolyl; pyrrolidinyl; oxazolyl; isoxazolyl; thiazolyl; isothiazolyl; imidazolyl; pyrazolyl; oxadiazolyl; thiadiazolyl; triazolyl; pyridyl; pyrazinyl; pyridazinyl; piperazinyl; pyrimidinyl; pyranyl; azetidinyl; morpholinyl; parathiazinyl; indolyl; indolinyl; benzo[*b*]furanyl; 2;3-dihydrobenzofuranyl; benzothienyl; 1H-indazolyl; benzimidazolyl; benzoxazolyl; benzisoxazolyl; benzthiazolyl; quinolinyl; isoquinolinyl; phthalazinyl; quinazolinyl; and quinoxalinyl; wherein (1.) said group R^{35} may be substituted upon any one or more carbon atoms thereof by 0 to 3 substituents R^{28} where R^{28} is as defined above, except that it is selected independently; (2.) said group R^{35} is substituted with respect to any one or more nitrogen atoms thereof that is not a point of attachment of said aryl or heterocyclic moiety, by 0 to 3 substituents $R^{13}{}_b$ where $R^{13}{}_b$ is as defined above, except that it is selected independently; and (3.) said group R^{35} with respect to any sulfur

atom thereof that is not a point of attachment of said heterocyclic moiety, is substituted by 0 or 2 oxygen atoms;

[R_{egion} β] is an alkyl bridging element of partial Formula (3.0.0):



(3.0.0)

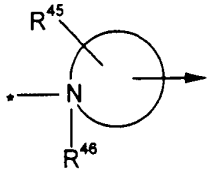
wherein:

–" * " is a symbol which represents the point of attachment of the moiety of partial Formula (3.0.0) to R_{egion} α;

–"→" is a symbol which represents the point of attachment of the moiety of partial Formula (3.0.0) to R_{egion} γ;

–R^{40} and R^{41} are both selected from the group consisting of hydrogen; (C_1-C_2) alkyl including dimethyl; hydroxy; and (C_1-C_3) alkoxy;

[R_{egion} γ] is an aza-monocyclic moiety of partial Formula (4.0.0):



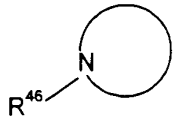
(4.0.0)

–wherein:

–" * " is a symbol which represents the point of attachment of the moiety of partial Formula (4.0.0) to R_{egion} β of the compound of Formula (I);

–"*→*" is a symbol representing a covalent bond attaching any carbon atom of said aza-monocyclic moiety of partial Formula (4.0.0) to R_{egion} δ;

–the moiety of partial Formula (4.0.1):



(4.0.1)

in partial Formula (4.0.0) represents a monocyclic heterocyclic group containing a total of from 4- to 7-members of which one said member is nitrogen, wherein said heterocyclic

group is a member independently selected from the group consisting essentially of azetidinyl; pyrrolidinyl; piperidinyl; and azepinyl;

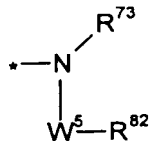
—R^{45} is absent or is a member independently selected from the group consisting essentially of (C_1-C_4)alkyl including dimethyl; (C_3-C_6)cycloalkyl; (C_1-C_4)alkoxy; CF_3; $-CO_2R^4$ where R^4 is as defined further above; oxo; -OH; cyano; $-C(=O)NR^4_aR^4_b$; $-NR^4_aR^4_b$; $-NR^4_aC(=O)R^4_b$; $-NR^4_aC(=O)OR^4_b$; $-NR^4_aS(=O)_pR^4_b$; $-S(=O)_pNR^4_aR^4_b$; (C_1-C_2)alkoxycarbonyl; (C_1-C_2)alkylcarbonyl; (C_1-C_2)alkylcarbonyloxy and (C_1-C_2)alkoxy(C_1-C_2)alkyl; it being understood that in the moiety of partial Formula (4.0.0) R^{45} is a substituent attached to a single carbon atom thereof; where:

—R^4_a and R^4_b are each independently selected from hydrogen and (C_1-C_2)alkyl;

—R^{46} is absent or is a member independently selected from the group consisting essentially of hydrogen; and (C_1-C_4)alkyl substituted by 0 or 1 substituent independently selected from (C_1-C_2)alkoxy and $-CO_2R^4$ where R^4 is as defined further above; and →O; it being understood that in the case where substituent R^{46} is present, that it results in said nitrogen atom and said moiety of partial Formula (4.0.0) is in quaternary form;

[R_{eglon} δ] is a member consisting of:

- an aryl and heterocyclyl-(substituted) amide, carbamate; or urea moiety of partial Formula (5.1.0):

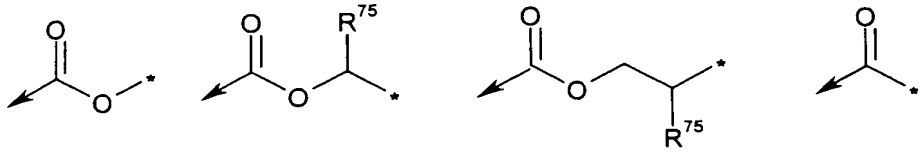


(5.1.0)

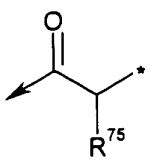
—wherein: the symbol " * " is as defined above;

—R^{73} is a member selected from the group consisting of hydrogen and (C_1-C_2)alkyl;

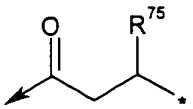
—W^5 is selected from the group consisting the moieties of partial Formulas (5.1.1) through (5.1.12):



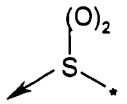
(5.1.1) (5.1.2) (5.1.3) (5.1.4)



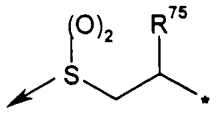
(5.1.5)



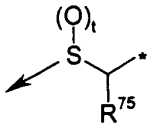
(5.1.6)



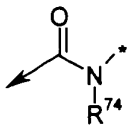
(5.1.7)



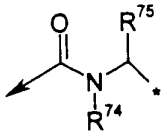
(5.1.8)



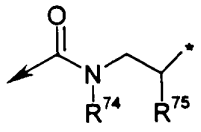
(5.1.9)



(5.1.10)



(5.1.11)



(5.1.12)

—wherein: the symbol: "→" indicates the point of attachment of the moiety W^5 represented by partial Formulas (5.1.1) through (5.1.12), inclusive, to the nitrogen atom in partial Formula (5.1.0), and the symbol: " * " indicates the point of attachment of the moiety W^5 to R^{82} as defined further below;

—R^{74} and R^{75} are each selected from the group consisting of hydrogen; $(C_1\text{-}C_2)$alkyl substituted by 0 or 1 substituent independently selected from OH; and $(C_1\text{-}C_2)$alkoxy; and

—R^{82} is a member selected from the group consisting of phenyl; cinnolinyl; furyl; thienyl; pyrrolyl; oxazolyl; isoxazolyl; thiazolyl; isothiazolyl; imidazolyl; imidazolinyl; pyrazolyl; pyrazolinyl; oxadiazolyl; thiadiazolyl; triazolyl; pyridyl; pyrazinyl; pyridazinyl; pyrimidinyl; parathiazinyl; indolyl; isoindolyl; indolinyl; benzo[*b*]furanyl; 2;3-dihydrobenzofuranyl; benzo[*b*]thiophenyl; 1H-indazolyl; benzimidazolyl; benzthiazolyl; quinolinyl; isoquinolinyl; phthalazinyl; quinazolinyl; quinoxalinyl; wherein:

——the aryl or heterocyclyl moiety is substituted by 0 to 3 substituents R^{78}, where:

——R^{78} is a member selected from the group consisting of oxo; -Cl; -F; -OH; $-(C_1\text{-}C_2)$alkyl; $-(C_1\text{-}C_3)$alkoxy; $-CF_3$; -CN; $-C(=O)OR^{79}$; $-C(=O)NR^{79}R^{80}$; $-NR^{79}R^{80}$; $-NR^{79}C(=O)R^{80}$; $-NR^{79}C(=O)OR^{80}$; $-NR^{79}S(=O)_2R^{80}$; and $-S(=O)_2NR^{79}R^{80}$, where:

———R^{79} and R^{80} are each a member independently selected from the group consisting of hydrogen; and $(C_1\text{-}C_4)$alkyl.

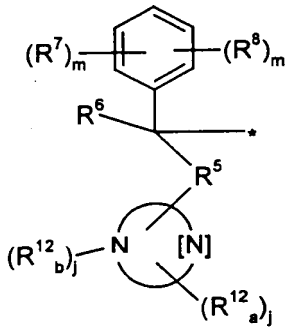
Attention is drawn to our copending application nos [attorney docket nos P60162WO and P60190WO].

An important aspect of the present invention is the limitation to R_{egion} δ. The copending cases relate to alternative limitations of Formula (I).

This invention also provides pharmaceutical formulations and dosage forms including as an active ingredient a compound of Formula I. Use of a compound of Formula I in manufacture of a formulation or dosage form and methods of treatment are also provided.

[R_{egion} α] is at the left-hand end of the CCR5 receptor modulator of the present invention. The region designated as R_{egion} α may comprise a moiety selected from several different classes of substituent components, all of which may be isosteres of each other.

The first class of R_{egion} α substituent components (under A.) are heterocyclyl phenylmethylene moieties as described further below. A preferred group of heterocyclyl phenylmethylene moiety embodiments (under A.1.) comprises the group consisting of hetero-phenylmethylene moieties of partial Formula (1.0.0),



(1.0.0)

The substituent R^5 is a member independently selected from the group consisting of a direct bond; -O-; -C(=O)-; -NR^4-; and-$S(=O)_p$-; where

R^4 is hydrogen or (C_1-C_2)alkyl.

The substituent R^6 is a member independently selected from the group consisting of hydrogen; (C_1-C_2)alkyl; (C_1-C_2)alkoxy; -C(=O)NH_2; -CN; and -OH. Most preferably R^6 is hydrogen and there is no substituent at this position.

Included within the partial Formula (1.0.0) are position isomer variations thereof that are not shown, but that arise where the optional substituents R^7 and R^8 are different. Substituents R^7 and R^8 are present once or twice or not at all, as indicated by their representation as: "$(R^7)_m$" and "$(R^8)_m$", where m is defined as being an integer selected from 0, 1, and 2. In the most preferred embodiments of the present invention, m is 0, although in alternative embodiments m is 1.

The substituents R^7 and R^8 comprise -F; -Cl; -CO_2R^4; -OH; -CN; -$CONR^4_aR^4_b$; -$NR^4_aR^4_b$-; -$NR^4_aC(=O)R^4_b$; -$NR^4_aC(=O)OR^4_b$; -$NR^4_aS(=O)_pR^4_b$; -$S(=O)_pNR^4_aR^4_b$; (C_1-C_4)alkyl including dimethyl, and (C_1-C_4)alkoxy wherein said alkyl and alkoxy are each independently substituted with 0 to 3 substituents independently selected from -F and -Cl;

$(C_1$-$C_2)$alkoxycarbonyl; $(C_1$-$C_2)$alkylcarbonyl; and $(C_1$-$C_2)$alkylcarbonyloxy. The substituents R^4_a and R^4_b, in turn, are selected from hydrogen and $(C_1$-$C_2)$alkyl. With regard to the R^7 and R^8 substituent groups, it is preferred that they are absent (m = 0); or that if they are present, that they be methyl; cyclopropyl, cyclobutyl; methoxy; -COOH; -OH; -F; -Cl; $-COO(C_1$-$C_2)$alkyl; or $-CF_3$. Of these choices, the more preferred substituent choices for R^7 and R^8 are that they are absent or that they are –F or Cl.

R^5 as defined by Formula (1.0.0) is preferably a direct bond. The moiety R^5 may alternatively be selected from –O-; -C(=O)-; $-NR^4$- where R^4 is hydrogen or $(C_1$-$C_2)$alkyl; and $-S(=O)_p$-.

In partial Formula (1.0.0), the presence of substituent R^{12}_a is determined by the subscript "j", which is an integer independently selected from 0, 1, and 2. Where j is 0, accordingly, the substituent R^{12}_a will be absent. Where j is 1 or 2, there may be one or two substituents R^{12}_a present, and these may be attached to any available carbon atom in partial Formula (1.0.0).

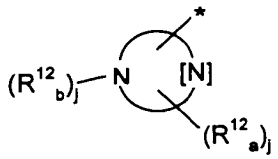
R^{12}_a is a member independently selected from the group consisting of hydrogen; -F; -Cl; $-CO_2R^4$ where R^4 is hydrogen or $(C_1$-$C_2)$alkyl as already defined above; -oxo; -OH; -CN; $-NH_2$; $-NH(C_1$-$C_2)$alkyl; $-N(C_1$-$C_2)_2$dialkyl; $-CF_3$; $(C_1$-$C_4)$alkyl; $(C_2$-$C_4)$alkenyl; $(C_1$-$C_4)$alkoxy; $(C_3$-$C_7)$cycloalkyl; and phenyl; wherein said alkyl, alkenyl, alkoxy, cycloalkyl and phenyl groups are substituted with 0 to 2 substituents R^9 wherein R^9 is a member independently selected from the group consisting of -F; -Cl; $-CO_2R^4$ where R^4 is hydrogen or $(C_1$-$C_2)$alkyl; -OH; cyano; $-CONR^4_aR^4_b$; $-NR^4_aR^4_b$; $-NR^4_aC(=O)R^4_b$; $-NR^4_aC(=O)OR^4_b$; $-NR^4_aS(=O)_pR^4_b$; $-S(=O)_pNR^4_aR^4_b$; $(C_1$-$C_4)$alkyl including dimethyl, and $(C_1$-$C_4)$alkoxy wherein said alkyl and alkoxy are each independently substituted with 0 to 3 substituents independently selected from F and Cl; $(C_1$-$C_2)$alkoxycarbonyl; $(C_1$-$C_2)$alkylcarbonyl; and $(C_1$-$C_2)$alkylcarbonyloxy.

Where a R^{12}_a substituent is present and consists of an alkyl, alkenyl, alkoxy, cycloalkyl or phenyl group, it may optionally be mono- or di-substituted in turn by a further substituent R^9, which is independently selected from the above-recited groups. This includes in particular $(C_1$-$C_4)$alkyl substituted with 1 to 3 substituents independently selected from F and Cl. Accordingly, the substituent $-CF_3$ is a preferred definition of R^9 in the compounds of partial Formula (1.0.0).

The R^{12}_b substituent is attached directly to the nitrogen atom of the heterocyclic group depicted in partial Formula (1.0.0), and its presence is determined by the subscript "j", which is an integer independently selected from 0, 1, and 2. Where j is 0, accordingly, the substituent R^{12}_b is absent. In that case that the nitrogen atom is attached by a covalent double bond to an adjacent atom in the heterocyclic group depicted in partial Formula (1.0.0). Where j is 1 or 2, there will be one or two substituents R^{12}_b attached to the nitrogen atom of the heterocyclic

group depicted in partial Formula (1.0.0). Where two such $R^{12}{}_b$ substituents are attached, the nitrogen atom is in quaternary form. The substituent $R^{12}{}_b$ is independently selected from the group consisting of hydrogen; $(C_1\text{-}C_4)$alkyl; $(C_2\text{-}C_4)$alkenyl; $(C_1\text{-}C_2)$alkoxy; $(C_3\text{-}C_7)$cycloalkyl; and phenyl; wherein said alkyl, alkenyl, alkoxy, cycloalkyl and phenyl are substituted with 0 to 2 substituents R^9 wherein R^9 has the same meaning as in R^9 defined above, except that it is selected independently therefrom.

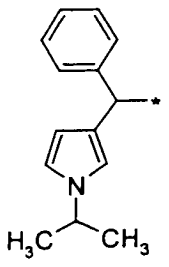
The group represented by partial Formula (1.0.1):



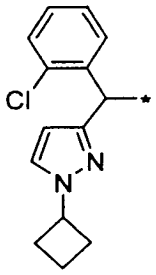
(1.0.1)

represents a monocyclic heterocyclic group, or a bicyclic benzo-fused ring system containing said heterocyclic group wherein said heterocyclic group contains a total of 5- or 6- members of which one or two of said members is nitrogen, the presence of the optional second nitrogen atom being represented by: "[N]"; wherein said heterocyclic group or ring system is selected from the group consisting of pyrrolyl; pyrazolyl; imidazolyl; pyridinyl; pyrazinyl; pyrimidinyl; pyridazinyl; indolyl; indazolinyl; benzimidazolyl; quinolinyl; *iso*-quinolinyl; and quinazolinyl.

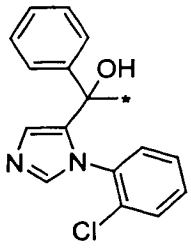
N-containing heterocyclic moieties of partial Formula (1.0.0) result in some of the following preferred embodiments of R_{egion} α, represented by partial Formulas (1.0.4) through (1.0.10), inclusive:



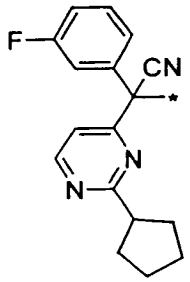
(1.0.4)



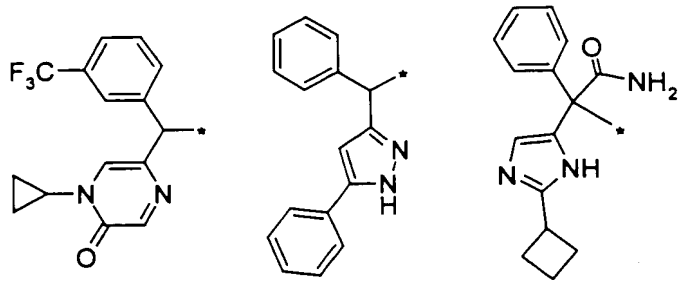
(1.0.5)



(1.0.6)

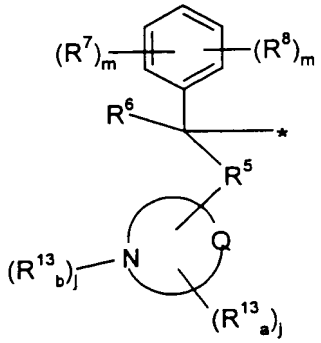


(1.0.7)



(1.0.8) (1.0.9) (1.0.10)

A further group of N-containing heterocyclic phenylmethylene moieties (under A.2 comprises several subgeneric groups within partial Formula (1.1.0):

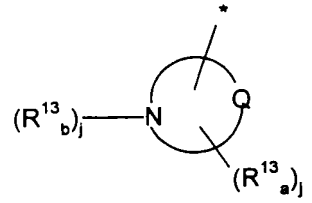


(1.1.0)

where the symbol " * " and R^5; R^6; R^7; R^8; j and m are as defined above;

and $R^{13}{}_a$ is a member selected from the group consisting of hydrogen; F; Cl; $-CO_2R^4$; oxo; -OH; CN; NH_2; $NH(C_1\text{-}C_2)$alkyl; $N(C_1\text{-}C_2)_2$dialkyl; $-CF_3$; $(C_1\text{-}C_4)$alkyl; $(C_2\text{-}C_4)$alkenyl; $(C_1\text{-}C_2)$alkoxy; $(C_3\text{-}C_7)$cycloalkyl; and phenyl; wherein said alkyl, alkenyl, alkoxy, cycloalkyl and phenyl are substituted with 0 to 2 substituents R^{11} wherein R^{11} is a member independently selected from the group consisting of F; Cl; $-CO_2R^4$; -OH; -CN; $-CONR^4{}_aR^4{}_b$; $-NR^4{}_aR^4{}_b$; $-NR^4{}_aC(=O)R^4{}_b$; $-NR^4{}_aC(=O)OR^4{}_b$; $-NR^4{}_aS(=O)_pR^4{}_b$; $-S(=O)_pNR^4{}_aR^4{}_b$; $(C_1\text{-}C_4)$alkyl including dimethyl, and $(C_1\text{-}C_4)$alkoxy wherein said alkyl and alkoxy are each independently substituted with 0 to 3 substituents independently selected from F and Cl; $(C_1\text{-}C_2)$alkoxycarbonyl; $(C_1\text{-}C_2)$alkylcarbonyl; and $(C_1\text{-}C_2)$alkylcarbonyloxy; and $R^{13}{}_b$ is selected from the group consisting of hydrogen; $(C_1\text{-}C_4)$alkyl; $(C_2\text{-}C_4)$alkenyl; $(C_1\text{-}C_2)$alkoxy; $(C_3\text{-}C_7)$cycloalkyl; $C(=O)(C_1\text{-}C_4)$alkyl; $S(=O)_2(C_1\text{-}C_4)$alkyl; and phenyl; wherein said alkyl, alkenyl, alkoxy, cycloalkyl and phenyl are substituted with 0 to 2 substituents R^{11} wherein R^{11} has the same meaning as in above, except that it is independently selected therefrom.

The moiety of partial Formula (1.1.1):

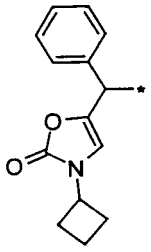


(1.1.1)

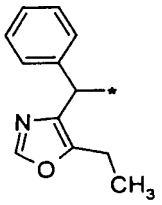
represents, *inter alia*, a monocyclic heterocyclic group containing a total of 5-members of which one said member is nitrogen and Q is selected from O and S

The heterocyclic group may be selected from the group consisting of oxazolyl; oxazolidinyl; *iso*xazolyl; thiazolyl; thiazolidinyl; *iso*-thiazolyl: morpholinyl and thiamorpholinyl.

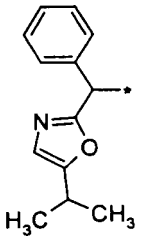
Moieties of partial Formula (1.1.0) containing the group of partial Formula (1.1.1) result in the following preferred embodiments of R_{egion} α, represented by partial Formulas (1.1.3) through (1.1.9):



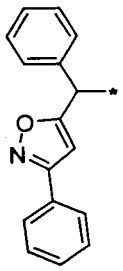
(1.1.3)



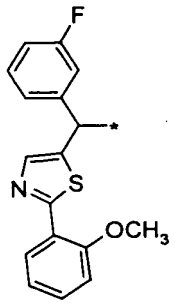
(1.1.4)



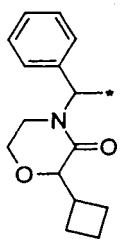
(1.1.5)



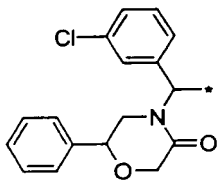
(1.1.6)



(1.1.7)



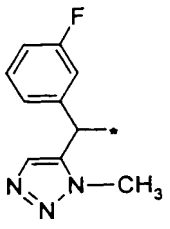
(1.1.8)



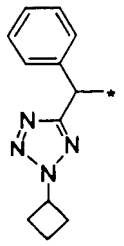
(1.1.9)

In alternative preferred embodiments the heterocyclic group may selected from the group consisting of triazolyl; triazinyl; tetrazolyl; oxadiazolyl; and thiadiazolyl.

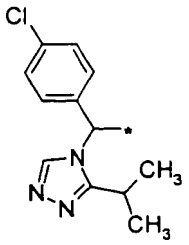
Further preferred embodiments of R_{egion} α, are represented by partial Formulas (1.1.20) through (1.1.24), inclusive:



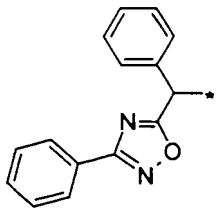
(1.1.20)



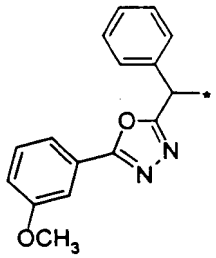
(1.1.21)



(1.1.22)



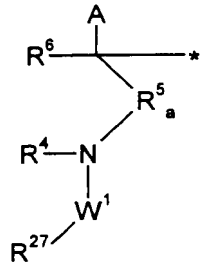
(1.1.23)



(1.1.24)

Another class of which R_{eglon} α moeities (under B) are (substituted)-amido-aryl or -heterocyclyl moieties which may be independently selected from several groups, as described in more detail below.

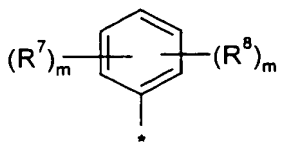
The first such class of (substituted)-amido-aryl or -heterocyclyl moieties of R_{eglon} α are those in which the amido-aryl or -heterocyclyl portion of the group is substituted by alkyl-, alkenyl-, or alkynyl, as represented by partial Formula (2.0.0)



(2.0.0)

where the symbol " * " and R^4 and R^6; and m, R^7 and R^8 in the further definition of A; are as defined in the partial formulas above, except that all of the above-recited substituents are selected independently.

The moiety A in partial Formula (2.0.0) is a member independently selected from the group consisting of several different classes of moieties, as discussed below. The first class represented by partial Formula (2.0.3) is a preferred embodiment of this invention.

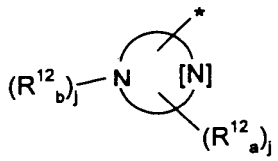


(2.0.3)

wherein the symbols R^7; R^8 and m are as defined in the partial formulas further above, except that all of the above-recited substituents are selected independently of their selection in

said partial formulas further above; and the symbol: " * " indicates the point of attachment of the moiety A to the other, remaining portions of partial Formula (2.0.0).

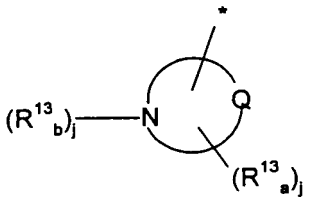
Further embodiments of moiety A are depicted by partial Formulas (2.0.4) and (2.0.5). Partial Formula (2.0.4) is:



(2.0.4)

which represents a monocyclic heterocyclic group, selected from the group consisting of pyrrolyl; pyrazolyl; imidazolyl; pyridinyl; pyrazinyl; and pyrimidinyl. It is noted that in the moiety of partial Formula (2.0.3), the symbols $R^{12}{}_a$ and $R^{12}{}_b$, and the subscript "j" which determines their presence, are as defined in the partial formulas further above, except that "j" is 0 or 1 and all of the above-recited substituents are selected independently of their selection further above; and the symbol: " * " indicates the point of attachment of the moiety A to the other, remaining portions of partial Formula (2.0.0).

Further embodiments of moiety A are depicted by partial Formula (2.0.5)



(2.0.5)

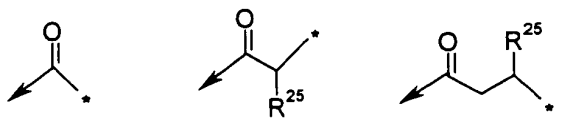
which represents a monocyclic heteroaromatic group containing a total of 5- members of which one said member is nitrogen and Q is selected from O and S where said S may optionally be in the sulfonate form, $-S(=O)_2$. Said heterocyclic group may be selected from the group consisting of oxazolyl; *iso*xazolyl; thiazolyl; and *iso*-thiazolyl; triazolyl; triazinyl; tetrazolyl; oxadiazolyl; and thiadiazolyl. It is noted that the symbols $R^{13}{}_a$ and $R^{13}{}_b$, and the subscript "j" which determines their presence, are as defined in the partial formulas further above, except that "j" is 0 or 1 and all of the above-recited substituents are selected independently of their selection in said partial formulas further above; and the symbol: " * " indicates the point of attachment of the moiety A to the other, remaining portions of partial Formula (2.0.0).

The group $R^5{}_a$ is selected from a direct bond; -C(=O)-; and $-S(=O)_2-$. In preferred embodiments of the present invention $R^5{}_a$ is a direct bond. It is provided, however, that where

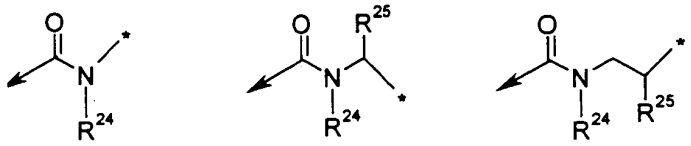
R^5_a is –CO- or -SO_2-, the divalent moiety W^1 is defined to additionally include the meaning of being a direct bond.

In partial Formula (2.0.0), R^{27} is a member selected from the group consisting of (C_1-C_6)alkyl; (C_2-C_6)alkenyl; and (C_2-C_6)alkynyl; wherein said alkyl, alkenyl, and alkynyl groups comprising R^{27} may be substituted with 0 to 3 substituents R^{28} where R^{28} is selected from the group consisting of F; Cl; oxo; hydroxy; (C_1-C_2)alkyl; (C_1-C_3)alkoxy; -C(=O)OR^{29}; C(=O)(C_1-C_4)alkyl; -S(=O)$_2$(C_1-C_4)alkyl; -C(=O)$NR^{29}R^{30}$; -$NR^{29}R^{30}$; -NR^{29}C(=O)R^{30}; -NR^{29}C(=O)OR^{30}; -NR^{29}S(=O)$_2R^{30}$; and -S(=O)$_2NR^{29}R^{30}$, where R^{29} and R^{30} are independently selected from hydrogen and (C_1-C_4)alkyl.

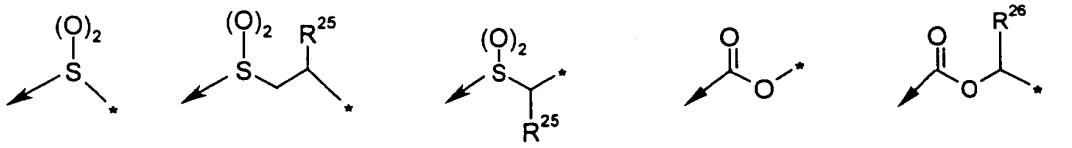
The moiety W^1 is a member independently selected from the group consisting of divalent moieties of partial Formulas (2.0.6) through (2.0.16), inclusive:



(2.0.6) (2.0.7) (2.0.8)



(2.0.19) (2.0.10) (2.0.11)

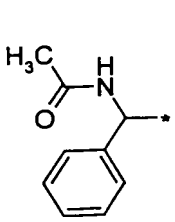


(2.0.12) (2.0.13) (2.0.14) (2.0.15) (2.0.16)

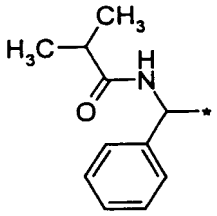
where the symbol: "→" indicates the point of attachment of the moiety W^1 to the nitrogen atom in partial Formula (2.0.0), and the symbol: " * " indicates the point of attachment of the moiety W^1 to the moiety R^{27} which represents the remaining portions of partial Formula (2.0.0); and R^{25} and R^{26} are each independently a member selected from the group consisting of hydrogen; (C_1-C_2)alkyl substituted by 0 or 1 substituent independently selected from F and OH; and (C_1-C_2)alkoxy.

The bridging element -N(R^4)-W^1- may alternatively constitute or contain several different functionalities. The first and most preferred of these is an amide functionality, which may be represented as: -NR^4-C(=O)-. Other functionality types include sulfonamido and ureido moieties within the scope of partial Formulas (2.0.6) through (2.0.16).

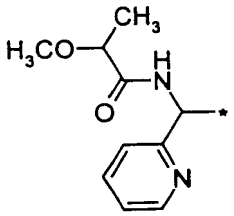
Preferred alkyl and alkenyl groups R^{27} include: methyl; ethyl; *iso*-propyl; *t*-butyl; and propenyl (allyl). These alkyl and alkenyl groups may be substituted by 0 to 3 substituents R^{28}. It is preferred that where a substituent is present that it be a single substituent independently selected from F; Cl; OH; CF_3; CH_3; OCH_3; CN; NH_2; $NH(CH_3)$; $N(CH_3)_2$; $NHCOCH_3$; and $NCH_3(COCH_3)$. Consequently, groups of partial Formula (2.0.0) which are preferred embodiments of the present invention constituting R_{egion} α include the following moieties of partial Formulas (2.0.30) through (2.0.36), inclusive:



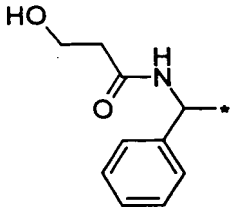
(2.0.30)



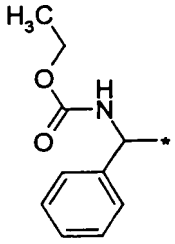
(2.0.31)



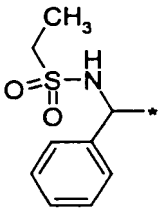
(2.0.32)



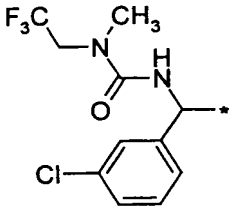
(2.0.33)



(2.0.34)

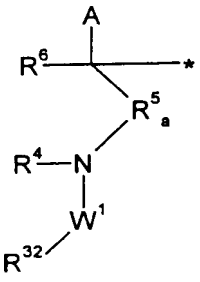


(2.0.35)



(2.0.36)

The second class of (substituted)-amido-aryl moieties comprising R_{egion} α are those in which the amido-aryl portion of the group is substituted by -(cycloalkyl) or -alkyl(cycloalkyl), as represented by partial Formula (2.1.0).

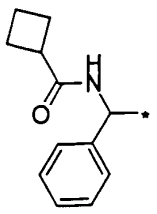


(2.1.0)

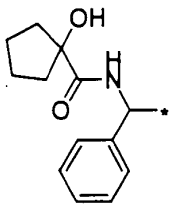
where; A; W^1; the symbol " * " and R^4; $R^5{}_a$; R^6; and m, R^7 and R^8 in the further definition of A; have the same meaning as set out in the partial formulas further above, except that all of the above-recited substituents are selected independently of their selection further

above. R^{32} is a member independently selected from the group consisting of -$-(CH_2)_n-(C_3\text{-}C_7)$cycloalkyl, where n is an integer selected from 0, 1, and 2; in the event n is 0, then the α-carbon atom of said $(C_3\text{-}C_7)$cycloalkyl may be substituted by $(C_1\text{-}C_4)$alkyl or phenyl, where said alkyl or phenyl may be substituted by 1, or 2 of CH_3, OCH_3, OH or NH_2; and in the event that n is 1 or 2, the resulting methylene or ethylene group may be substituted by of F; Cl; CN; NH_2; $N(CH_3)_2$; OH; OCH_3; $(C_1\text{-}C_4)$alkyl; or phenyl. It will also be further noted that the basic $(C_3\text{-}C_7)$cycloalkyl group comprising R^{32} may also be substituted by 0 to 3 substituents R^{28} where R^{28} has the same meaning as defined further above with respect to substituents for group R^{27} under partial Formula (2.0.0), but independently selected therefrom.

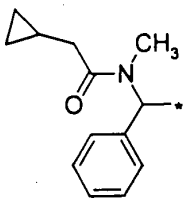
Representative cycloalkyl and alkylcycloalkyl groups within the scope of R^{32} include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl; cyclopropylmethyl; cyclobutylethyl; cyclopentylpropmethyl; and cyclopentylmethyl. More preferred single substituents for these cycloalkyl and alkylcycloalkyl groups include F, Cl, and CN, especially OH; OCH_3; and NH_2. Accordingly, groups of partial Formula (2.1.0) which are preferred embodiments of R_{egion} α include partial Formulas (2.1.3) through (2.1.10).



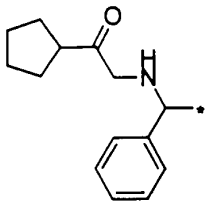
(2.1.3)



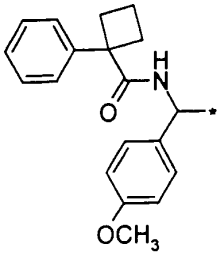
(2.1.4)



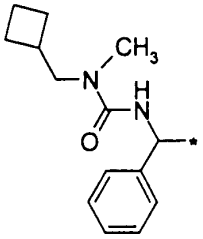
(2.1.5)



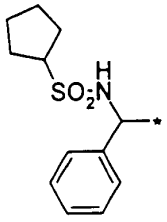
(2.1.6)



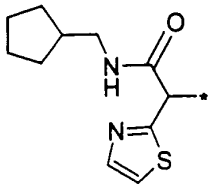
(2.1.7)



(2.1.8)

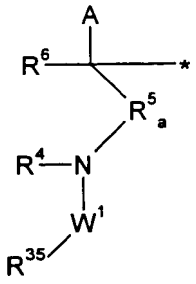


(2.1.9)



(2.1.10)

The third class of (substituted)-amido-aryl moieties of R_{egion} α are those in which the amido-aryl portion of the group is substituted by aryl- and heterocyclyl-substituted-amido-aryl moieties of partial Formula (2.2.0).



(2.2.0)

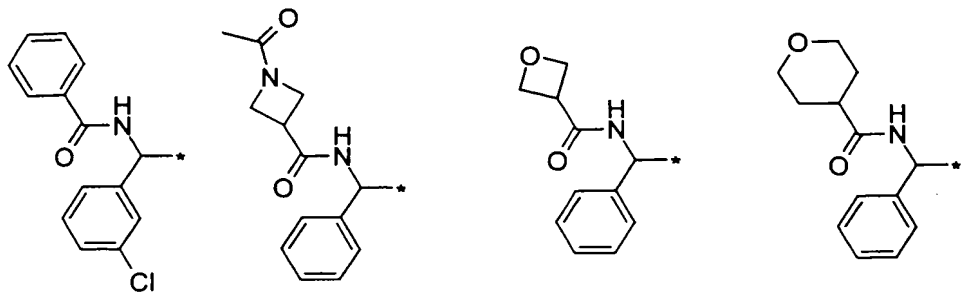
where A; W^1; the symbol " * " and R^4; $R^5{}_a$; R^6; and m, R^7 and R^8 in the definition of A; have the same meaning as set out above, except that all of the above-recited substituents are selected independently.

The moiety R^{35} may be selected from the group consisting of phenyl; furyl; tetrahydropyranyl; tetrahydrofuranyl; oxetanyl; thienyl; pyrrolyl; pyrrolidinyl; oxazolyl; isoxazolyl; thiazolyl; isothiazolyl; imidazolyl; imidazolinyl; pyrazolyl; pyrazolinyl; oxadiazolyl; thiadiazolyl; triazolyl; pyridyl; pyrazinyl; pyridazinyl; piperazinyl; pyrimidinyl; pyranyl; azetidinyl; morpholinyl; parathiazinyl; indolyl; isoindolyl; 3H-indolyl; indolinyl; benzo[*b*]furanyl; 2;3-dihydrobenzofuranyl; benzothienyl; 1H-indazolyl; benzimidazolyl; benzoxazolyl; benzisoxazolyl; benzthiazolyl; benzoxdiazolyl; quinolinyl; isoquinolinyl; phthalazinyl; quinazolinyl; and quinoxalinyl.

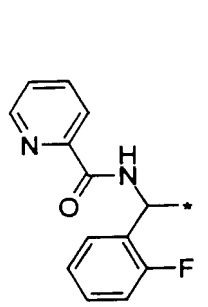
Preferred meanings of R^{35} are phenyl; pyrrolyl; oxazolyl; imidazolyl; pyridinyl; pyrimidinyl; triazolyl; indolyl; benzimidazolyl; benzotriazolyl; quinolinyl; thienyl; furfuryl; benzofuranyl; thiazolyl; oxazolyl; isoxazolyl; oxadiazolyl; and benzoxazolyl; and benzoxadiazolyl. Most preferred are tetrahydropyranyl; oxetanyl; azetidinyl and tetrahydrofuranyl. Group R^{35} may be substituted by 3 substituents R^{28} where R^{28} has the same meaning as defined above but selected independently.

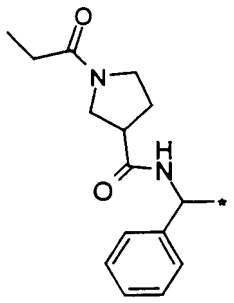
Alternative aryl and heterocyclyl groups falling within the scope of R^{35} include phenyl; pyrrolyl; imidazolyl; pyridyl; oxazolyl; furyl; and benzofuranyl. Preferred single or double substituents for these groups include -CN; -F; -Cl; $-CONH_2$; $-CH_3$; $-CF_3$; and $-OCH_3$.

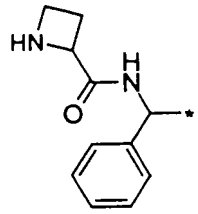
Accordingly, groups of partial Formula (2.2.0) which are preferred embodiments of R_{egion} α include partial Formulas (2.2.3) through (2.2.14)

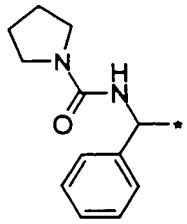


(2.2.3) (2.2.4) (2.2.5) (2.2.6)

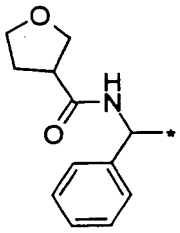


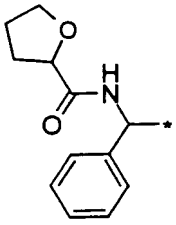


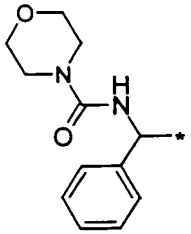


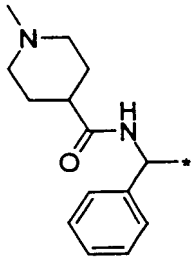


(2.2.7) (2.2.8) (2.2.9) (2.2.10)





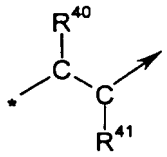




(2.2.11) (2.2.12) (2.2.13) (2.2.14)

[R_{egion} β] may be considered to be to the left-hand end of the molecule of the present invention as depicted, and comprises a bridging element between R_{egion} α described above, and R_{egion} γ described below.

The alkyl bridging element of R_{egion} β comprises a moiety of partial Formula (3.0.0):

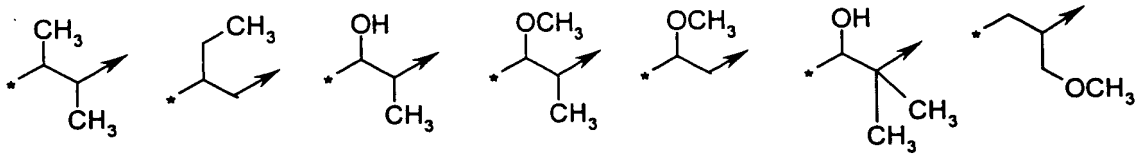


(3.0.0)

where the symbol " * " represents the point of attachment of the alkyl bridging element moiety of partial Formula (3.0.0) to R_{egion} α of the antagonist compound of Formula (I); and the symbol "→" represents the point of attachment of the alkyl bridging element moiety of partial Formula (3.0.0) to R_{egion} γ of the antagonist compound of Formula (I). Substituents R^{40} and R^{41} are both independently selected from the group consisting of hydrogen; (C_1-C_2) alkyl including dimethyl; hydroxy; and (C_1-C_3) alkoxy; provided that only one of R^{40} and R^{41} may be (C_1-C_3) alkoxy or hydroxy, the other one of R^{40} or R^{41} being selected from hydrogen and (C_1-C_2) alkyl including dimethyl.

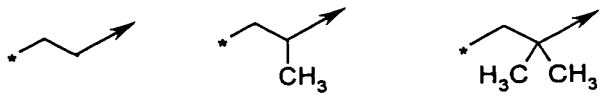
Accordingly, R^{40} and R^{41} may be hydrogen; methyl; ethyl; dimethyl, *i.e.*, two methyl groups joined to the single carbon atom to which R^{40} or R^{41} is attached; hydroxy; methoxy; ethoxy; or propoxy.

Some representative embodiments of the alkyl bridging element of partial Formula (3.0.0) include the following moieties of partial Formulas (3.0.1) through (3.0.7), inclusive:



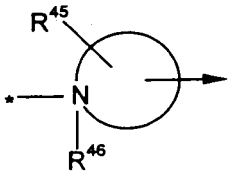
(3.0.1) (3.0.2) (3.0.3) (3.0.4) (3.0.5) (3.0.6) (3.0.7)

In the most preferred embodiments of the antagonist compounds of the present invention, both R^{40} and R^{41} are hydrogen, and the alkyl bridging element of partial Formula (3.0.0) is unsubstituted ethylene. In preferred embodiments a single methyl, hydroxy, or methoxy substituent may be present, resulting in alkyl bridging elements such as those of partial Formulas (3.0.8) through (3.0.10):



(3.0.8) (3.0.9) (3.0.10)

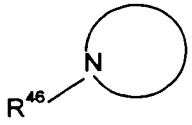
$\underline{[R_{egion}\ \gamma]}$ comprises a member selected from the group consisting of a moiety of partial Formula (4.0.0):



(4.0.0)

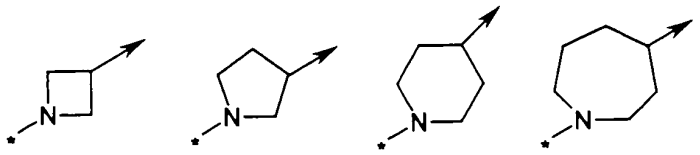
where " * " is a symbol representing the point of attachment of the aza-monocyclic moiety of partial Formula (4.0.0) to $R_{egion}\ \beta$; and "→" is a symbol representing the point of attachment to $R_{egion}\ \delta$. It will be noted that in the moieties of partial Formula (4.0.0) the nitrogen atom covalently bonds said heterocyclic moieties to $R_{egion}\ \beta$.

The heterocyclic moiety of partial Formula (4.0.1):



(4.0.1)

constituting a part of partial Formula (4.0.0) represents a monocyclic heterocyclic group containing a total of from 4- to 7-members of which one said member is nitrogen, wherein said heterocyclic group is a member independently selected from the group consisting essentially of azetidinyl; pyrrolidinyl; piperidinyl; and azepinyl, which may also be referred to as homopiperidinyl. With respect to the moieties of partial Formula (4.0.0) which define $R_{region\ \chi}$ then, there is included the following groups represented by partial Formulas (4.0.2) through (4.0.5):

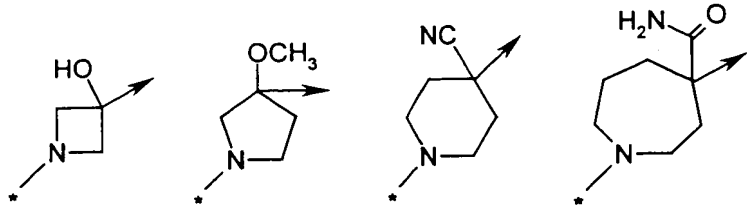


(4.0.2) (4.0.3) (4.0.4) (4.0.5)

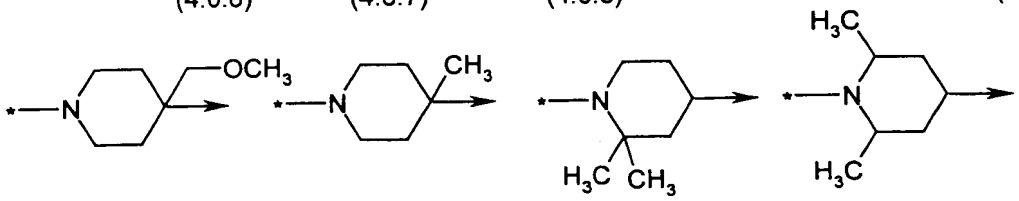
The above-defined moieties of partial Formula (4.0.0) are optionally mono-substituted by R^{45} where R^{45} is absent or is a member independently selected from the group consisting essentially of $(C_1\text{-}C_4)$alkyl including dimethyl; $(C_3\text{-}C_6)$cycloalkyl; $(C_1\text{-}C_4)$alkoxy; $-CF_3$; $-CO_2R^4$ where R^4 is as defined further above; oxo; -OH; -CN; $-C(=O)NR^4{}_aR^4{}_b$; $-NR^4{}_aR^4{}_b$; $-NR^4{}_aC(=O)R^4{}_b$; $-NR^4{}_aC(=O)OR^4{}_b$; $-NR^4{}_aS(=O)_pR^4{}_b$; $-S(=O)_pNR^4{}_aR^4{}_b$ where $R^4{}_a$ and $R^4{}_b$ are are each independently selected from hydrogen; $(C_1\text{-}C_2)$alkyl; $(C_1\text{-}C_2)$alkoxycarbonyl; $(C_1\text{-}C_2)$alkylcarbonyl; $(C_1\text{-}C_2)$alkylcarbonyloxy and $(C_1\text{-}C_2)$alkoxy$(C_1\text{-}C_2)$alkyl. It will be understood that in the moieties of partial Formula (4.0.0), the substituent R^{45} is attached to a single carbon atom of the above-above-described heterocyclic group. It will be further understood that where R^{45} is defined as (C_1)alkyl, the methyl substituent may occur twice on a single carbon atom of the heterocyclic group, *i.e.*, be a dimethyl substituent.

The substituent group R^{46} is absent or is a member independently selected from the group consisting essentially of hydrogen; $(C_1\text{-}C_4)$alkyl substituted by 0 or 1 substituent independently selected from $(C_1\text{-}C_2)$alkoxy and $-CO_2R^4$ where R^4 is as defined further above; and $\rightarrow O$. It will be appreciated that in the case where substituent R^{46} is selected to be other than absent, that it will result in said nitrogen atom and said moiety of partial Formula (4.0.0) being in quaternary form. However, generally the quaternary forms of the compounds of the present invention are less preferred than their non-quaternary counterparts, although the skilled artisan can readily foresee that some particular embodiment may have more advantageous properties in its quaternary form than in its non-quaternary form.

Although it is preferred that the moieties of partial Formula (4.0.0) remain unsubstituted, *i.e.*, that R^{45} be absent, some examples of substituted moieties which are included within the scope of preferred embodiments of the present invention are those depicted in partial Formulas (4.0.6) through (4.0.13), inclusive:



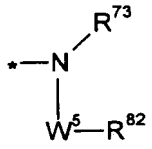
(4.0.6) (4.0.7) (4.0.8) (4.0.9)



(4.0.10) (4.0.11) (4.0.12) (4.0.13)

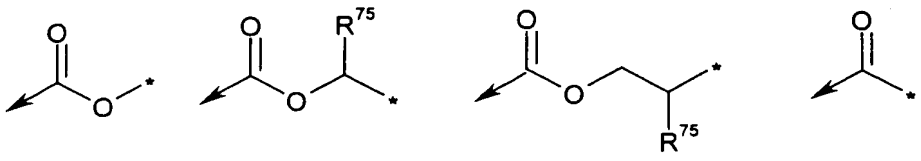
[R_{egion} δ] constitutes the right-hand end of the compounds of Formula (I) and is attached directly to R_{egion} γ described above.

R_{egion} δ consists of the compounds of Formula (I) which comprise subclasses having aryl and heterocyclyl-(substituted)-amides, carbamates or ureas of partial Formula (5.1.0):

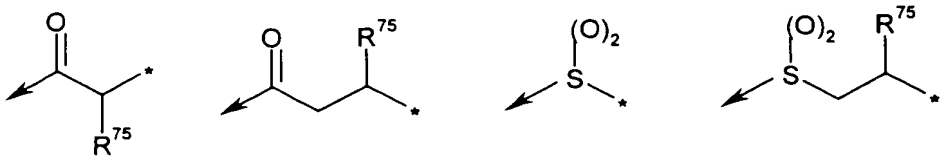


(5.1.0)

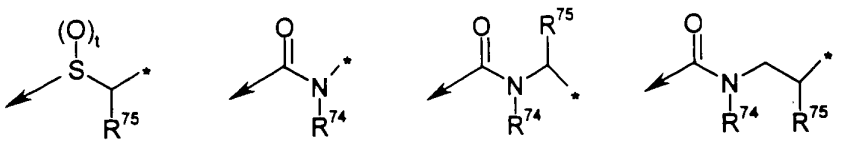
where the symbol " * " has the same meaning as defined further above; R^{73} is hydrogen or (C_1-C_2)alkyl; and W^5 is selected from the moieties of partial Formulas (5.1.1) through (5.1.12), inclusive:



(5.1.1) (5.1.2) (5.1.3) (5.1.4)



(5.1.5) (5.1.6) (5.1.7) (5.1.8)



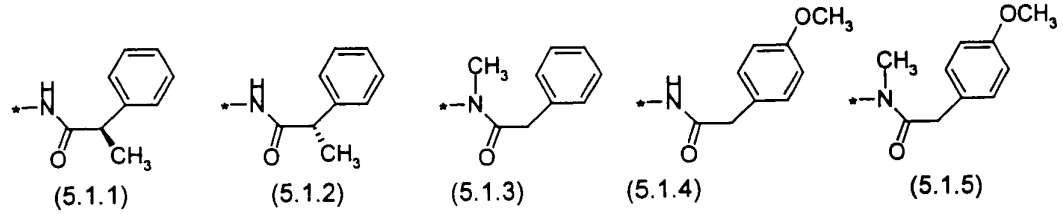
(5.1.9) (5.1.10) (5.1.11) (5.1.12)

where the symbol: "→" indicates the point of attachment of the moiety W^5 represented by partial Formulas (5.1.1) through (5.1.12) to the nitrogen atom in partial Formula (5.1.0), and the symbol: " * " indicates the point of attachment of the moiety W^5 to R^{82}. The substituents R^{74} and R^{75} are independently selected from hydrogen; (C_1-C_2)alkyl substituted by 0 or 1 substituent independently selected from OH; and (C_1-C_2)alkoxy.

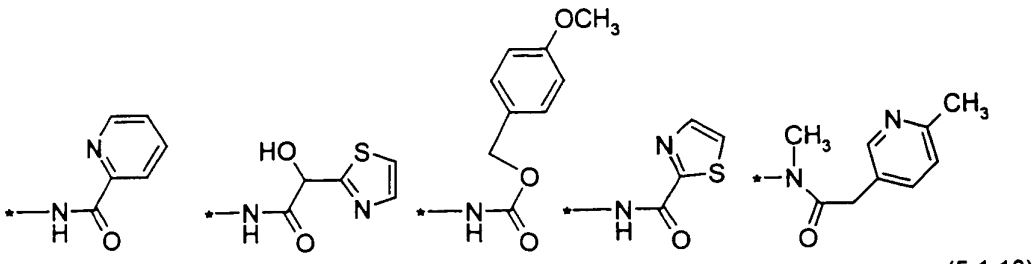
The group R^{82} may be selected from phenyl; cinnolinyl; furyl; thienyl; pyrrolyl; oxazolyl; isoxazolyl; thiazolyl; isothiazolyl; imidazolyl; imidazolinyl; pyrazolyl; pyrazolinyl; oxadiazolyl; thiadiazolyl; triazolyl; pyridyl; pyrazinyl; pyridazinyl; pyrimidinyl; parathiazinyl; indolyl; isoindolyl; indolinyl; benzo[*b*]furanyl; 2;3-dihydrobenzofuranyl; benzo[*b*]thiophenyl; 1H-indazolyl; benzimidazolyl; benzthiazolyl; quinolinyl; isoquinolinyl; phthalazinyl; quinazolinyl; and quinoxalinyl. The aryl or heterocyclyl groups comprising R^{82} may be substituted with 0 to 3 substituents R^{78}, where R^{78} is selected from oxo; -Cl; -F; -OH; (C_1-C_2)alkyl; (C_1-C_3)alkoxy; $-CF_3$; -CN; $-C(=O)OR^{79}$; $-C(=O)NR^{79}R^{80}$; $-NR^{79}R^{80}$; $-NR^{79}C(=O)R^{80}$; $-NR^{79}C(=O)OR^{80}$; $-NR^{79}S(=O)_2R^{80}$; and $-S(=O)_2NR^{79}R^{80}$, where R^{79} and R^{80} are each hydrogen or (C_1-C_4)alkyl.

Preferred groups of Formula (5.1.0) include ureas and amides. Carbamates are most preferred.

Accordingly, preferred embodiments of the compounds of the present invention include partial Formulas (5.1.1) through (5.1.10):



(5.1.1) (5.1.2) (5.1.3) (5.1.4) (5.1.5)



(5.1.6) (5.1.7) (5.1.8) (5.1.9) (5.1.10)

The compounds of the present invention may be utilized in the form of acids, esters, or other chemical derivatives. It is also within the scope of the present invention to utilize those compounds in the form of pharmaceutically acceptable salts derived from various organic and inorganic acids and bases in accordance with procedures well known in the art. The expression "pharmaceutically acceptable salt" as used herein is intended to mean an active ingredient comprising a compound of Formula (I) utilized in the form of a salt thereof, especially where said salt form confers on said active ingredient improved pharmacokinetic properties as compared to the free form of said active ingredient or other previously disclosed salt form.

A pharmaceutically acceptable salt form of said active ingredient may also initially confer a desirable pharmacokinetic property on said active ingredient which it did not previously possess, and may even positively affect the pharmacodynamics of said active ingredient with respect to its therapeutic activity in the body.

The pharmacokinetic properties of said active ingredient which may be favorably affected include, *e.g.*, the manner in which said active ingredient is transported across cell membranes, which in turn may directly and positively affect the absorption, distribution, biotransformation or excretion of said active ingredient. While the route of administration of the pharmaceutical composition is important and various anatomical, physiological and pathological factors can critically affect bioavailability, the solubility of said active ingredient is usually dependent upon the character of the particular salt form thereof which it utilized. Further, an aqueous solution may provide the most rapid absorption of an active ingredient into the body of a patient being treated, while lipid solutions and suspensions, as well as solid dosage forms, may result in less rapid absorption. Oral ingestion of said active ingredient is the most preferred route of administration for reasons of safety, convenience, and economy, but absorption of such an oral dosage form can be adversely affected by physical characteristics such as polarity, emesis caused by irritation of the gastrointestinal mucosa, destruction by digestive enzymes and low pH, irregular absorption or propulsion in the presence of food or other drugs, and metabolism by enzymes of the mucosa, the intestinal flora, or the liver. Formulation of said active ingredient into different pharmaceutically acceptable salt forms may be effective in overcoming or alleviating one or more of the above-recited problems encountered with absorption of oral dosage forms.

Well-known pharmaceutically acceptable salts include, but are not limited to acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, besylate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecysulfate, ethanesulfonate, fumarate, glucoheptanoate, gluconate, glycerophosphate, hemisuccinate,

hemisulfate, heptanoate, hexanoate, hippurate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, isethionate, lactate, lactobionate, maleate, mandelate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oxalate, oleate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphonate, picrate, pivalate, propionate, salicylate, sodium phosphate, stearate, succinate, sulfate, sulfosalicylate, tartrate, thiocyanate, thiomalate, tosylate, and undecanoate.

Base salts of the compounds of the present invention include, but are not limited to ammonium salts; alkali metal salts such as sodium and potassium; alkaline earth metal salts such as calcium and magnesium; salts with organic bases such as dicyclohexylamine, meglumine, N-methyl-D-glucamine, tris-(hydroxymethyl)-methylamine (tromethamine), and salts with amino acids such as arginine, lysine, *etc.* Compounds of the present invention which comprise basic nitrogen-containing groups may be quaternized with such agents as (C_1-C_4) alkyl halides, *e.g.*, methyl, ethyl, *iso*-propyl and *tert*-butyl chlorides, bromides and iodides; di(C_1-C_4) alkyl sulfate, *e.g.*, dimethyl, diethyl and diamyl sulfates; (C_{10}-C_{18}) alkyl halides, *e.g.*, decyl, dodecyl, lauryl, myristyl and stearyl chlorides, bromides and iodides; and aryl-(C_1-C_4) alkyl halides, *e.g.*, benzyl chloride and phenethyl bromide. Such salts permit the preparation of both water-soluble and oil-soluble compounds of the present invention.

Among the above-recited pharmaceutical salts those which are preferred include, but are not limited to acetate, besylate, citrate, fumarate, gluconate, hemisuccinate, hippurate, hydrochloride, hydrobromide, isethionate, mandelate, meglumine, nitrate, oleate, phosphonate, pivalate, sodium phosphate, stearate, sulfate, sulfosalicylate, tartrate, thiomalate, tosylate, and tromethamine.

Multiple salts forms are included within the scope of the present invention where a compound of the present invention contains more than one group capable of forming such pharmaceutically acceptable salts. Examples of typical multiple salt forms include, but are not limited to bitartrate, diacetate, difumarate, dimeglumine, diphosphate, disodium, and trihydrochloride.

The compounds of this invention can be administered alone but will generally be administered in admixture with one or more suitable pharmaceutical excipients, diluents or carriers selected with regard to the intended route of administration and standard pharmaceutical practice.

For example, the compounds of the formula (I) can be administered orally or sublingually in the form of tablets, capsules, ovules, elixirs, solutions or suspensions, which may contain flavouring or colouring agents, for immediate or controlled release applications.

Such tablets may contain excipients such as microcrystalline cellulose, lactose, sodium citrate, calcium carbonate, dicalcium phosphate and glycine, disintegrants such as starch (preferably corn, potato or tapioca starch), alginic acid and certain complex silicates, and granulation binders such as polyvinylpyrrolidone, sucrose, gelatin and acacia. Additionally, lubricating agents such as magnesium stearate, sodium lauryl sulfate and talc may be included.

Solid compositions of a similar type may also be employed as fillers in gelatin capsules. Preferred excipients in this regard include lactose or milk sugar as well as high molecular weight polyethylene glycols. For aqueous suspensions and/or elixirs, the compounds of the formula (I) may be combined with various sweetening or flavouring agents, colouring matter or dyes, with emulsifying and/or suspending agents and with diluents such as water, ethanol, propylene glycol and glycerin, and combinations thereof.

The compounds of the formula (I) can also be injected parenterally, for example, intravenously, intraperitoneally, intrathecally, intraventricularly, intrasternally, intracranially, intramuscularly or subcutaneously, or they may be administered by infusion techniques. They are best used in the form of a sterile aqueous solution which may contain other substances, for example, enough salts or glucose to make the solution isotonic with blood. The aqueous solutions should be suitably buffered (preferably to a pH of from 3 to 9), if necessary. The preparation of suitable parenteral formulations under sterile conditions is readily accomplished by standard pharmaceutical techniques well known to those skilled in the art.

For oral and parenteral administration to human patients, the daily dosage level of the compounds of the formula (I) will usually be from 1 microgram/kg to 25 mg/kg (in single or divided doses).

Thus tablets or capsules of the compound of the formula (I) may contain from 0.05 mg to 1.0 g of active compound for administration singly or two or more at a time, as appropriate. The physician in any event will determine the actual dosage which will be most suitable for any individual patient and it will vary with the age, weight and response of the particular patient. The above dosages are exemplary of the average case. There can, of course, be individual instances where higher or lower dosage ranges are merited and such are within the scope of this invention.

The compounds of formula (I) can also be administered intranasally or by inhalation and are conveniently delivered in the form of a dry powder inhaler or an aerosol spray presentation from a pressurised container or a nebuliser with the use of a suitable propellant, eg dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, a hydrofluoroalkane such as 1,1,1,2-tetrafluorethane (HFA 134a), carbon dioxide or other suitable gas. In the case of a pressurised aerosol, the dosage unit may be determined by

providing a valve to deliver a metered amount. The pressurised container or nebuliser may contain a solution or suspension of the active compound, eg using a mixture of ethanol and the propellant as the solvent, which may additional contain a lubricant, eg sorbitan trioleate. Capsules and cartridges (made, for example, from gelatin) for use in an inhaler or insufflator may be formulated to contain a powder mix of a compound of the formula (I) and a suitable powder base such as lactose or starch.

Aerosol or dry powder formulations are preferably arranged so that each metered dose or "puff" contains from 20 μg to 20 mg of a compound of the formula (I) for delivery to the patient. The overall daily dose with an aerosol will be in the range of from 20 μg to 20 mg which may be administered in a single dose or, more usually, in divided doses throughout the day.

Alternatively, the compounds of the formula (I) can be administered in the form of a suppository or pessary, or they may be applied topically in the form of a lotion, solution, cream, ointment or dusting powder. The compounds of the formula (I) may also be transdermally administered by the use of a skin patch. They may also be administered by the ocular route, particularly for treating neurological disorders of the eye.

For ophthalmic use, the compounds can be formulated as micronised suspensions in isotonic, pH adjusted, sterile saline, or, preferably, as solutions in isotonic, pH adjusted, sterile saline, optionally in combination with a preservative such as benzylalkonium chloride. Alternatively, they may be formulated in an ointment such as petrolatum.

For application topically to the skin, the compounds of the formula (I) can be formulated as a suitable ointment containing the active compound suspended or dissolved in, for example, a mixture with one or more of the following: mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene polyoxypropylene compound, emulsifying wax and water. Alternatively, they can be formulated as a suitable lotion or cream, suspended or dissolved in, for example, a mixture of one or more of the following: mineral oil, sorbitan monostearate, a polyethylene glycol, liquid paraffin, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benyl alcohol and water.

The compounds of Formula (I) are described herein as possessing biological activity such that they are able to modulate CCR5 chemokine receptor activity and consequent or associated pathogenic processes subsequently mediated by the CCR5 receptor and its ligands. The expression "modulate CCR5 chemokine receptor activity" as used herein is intended to refer to manipulation of the basic physiological processes and agencies which involve CCR5 chemokine receptors and their ligands. Included within the scope of this intended meaning are all types and subtypes of CCR5 receptors, in whatever tissues of a particular patient they are found, and in or on whatever components of the cells comprising

those tissues they may be located. Most commonly, CCR5 receptors are situated on the cell membranes of particular cell types such as monocytes. CCR5 receptors participate in and define, along with various endogenous ligands to which they are naturally bound, signaling pathways which control important cellular and tissue functions by means of the influence which they exert on the movement of agents such as the chemokines, into and out of those cells and tissues.

The basic functioning of the CCR5 receptors and their ligands may be modulated in a number of ways, and the scope of the present invention is not limited in that regard to any particular existing or hypothesized pathway or process. Thus, included within the intended meaning of modulation of CCR5 chemokine receptor activity, is the use of synthetically derived modulators introduced into a patient being treated, such as the compounds of Formula (I) described herein. These exogenous agents may modulate CCR5 receptor activity by such well known mechanisms as competitive binding in which the natural ligands are displaced and their inherent functions disrupted. However, the present invention is not limited to any such specific mechanism or mode of action. Thus, "modulation" as used herein is intended to encompass preferably agonism, but also antagonism, partial agonism and/or partial antagonism. Correspondingly, the term "therapeutically effective amount" means the amount of the subject compound that will elicit the biological or medical response of a tissue, system, animal or human that is being sought.

The term "patient" in this specification refers particularly to humans. However the compounds, methods and pharmaceutical compositions of the present invention may be used in the treatment of animals.

Further included within the scope of the present invention are metabolites or residues of the compounds of Formula (I) which possess biological activity such that they are able to modulate CCR5 chemokine receptor activity and consequent or associated pathogenic processes subsequently mediated by the CCR5 receptor and its ligands. Once synthesized, the CCR5 chemokine receptor modulating activities and specificities of the compounds of Formula (I) according to the present invention may be determined using *in vitro* and *in vivo* assays which are described in detail further below.

The desirable biological activity of the compounds of Formula (I) may also be improved by appending thereto appropriate functionalities which enhance existing biological properties of the compound, improve the selectivity of the compound for the existing biological activities, or add to the existing biological activities further desirable biological activities. Such modifications are known in the art and include those which increase biological penetration into a given biological system, *e.g.*, blood, the lymphatic system, and central nervous system;

increase oral availability; increase solubility to allow administration by injection; alter metabolism; and alter the rate of excretion of the compound of Formula (I).

The dosage and dose rate of the compounds of Formula (I) effective for treating or preventing diseases and conditions in a patient which are mediated by or associated with modulation of CCR5 chemokine receptor activity as described herein, as well as for favorably affecting the outcome thereof in said patient, in accordance with the methods of treatment of the present invention comprising administering to said patient a therapeutically effective amount of a compound of Formula (I), will depend on a variety of factors such as the nature of the active ingredient, the size of the patient, the goal of the treatment, the nature of the pathology being treated, the specific pharmaceutical composition used, the concurrent treatments that the patient may be subject to, and the observations and conclusions of the treating physician.

Generally, however, the effective therapeutic dose of a compound of Formula (I) which will be administered to a patient will be between about 10 μg (0.01 mg)/kg and about 60.0 mg/kg of body weight per day, preferably between about 100 μg (0.1 mg)/kg and about 10 mg/kg of body weight per day, more preferably between about 1.0 mg/kg and about 6.0 mg/kg of body weight per day, and most preferably between about 2.0 mg/kg and about 4.0 mg/kg of body weight per day of the active ingredient of Formula (I).

Included within the scope of the present invention are embodiments comprising coadministration of, and compositions which contain, in addition to a compound of the present invention as active ingredient, additional therapeutic agents and active ingredients. Such multiple drug regimens, often referred to as combination therapy, may be used in the treatment and prevention of any of the diseases or conditions mediated by or associated with CCR5 chemokine receptor modulation, particularly infection by human immunodeficiency virus, HIV. The use of such combinations of therapeutic agents is especially pertinent with respect to the treatment and prevention of infection and multiplication within a patient in need of treatment or one at risk of becoming such a patient, of the human immunodeficiency virus, HIV, and related pathogenic retroviruses. The ability of such retroviral pathogens to evolve within a relatively short period of time into strains resistant to any monotherapy which has been administered to said patient is well known in the technical literature.

In addition to the requirement of therapeutic efficacy which may necessitate the use of active agents in addition to the CCR5 chemokine receptor modulating compounds of Formula (I), there may be additional rationales which compel or highly recommend the use of combinations of drugs involving active ingredients which represent adjunct therapy, *i.e.*, which complement and supplement the function performed by the CCR5 chemokine receptor modulating compounds of the present invention. Such supplementary therapeutic agents

used for the purpose of auxiliary treatment include drugs which, instead of directly treating or preventing a disease or condition mediated by or associated with CCR5 chemokine receptor modulation, treat diseases or conditions which directly result from or indirectly accompany the basic or underlying CCR5 chemokine receptor modulated disease or condition. For example, where the basic CCR5 chemokine receptor modulated disease or condition is HIV infection and multiplication, it may be necessary or at least desirable to treat opportunistic infections, neoplasms, and other conditions which occur as the result of the immune-compromised state of the patient being treated. Other active agents may be used with the compounds of Formula (I), *e.g.*, in order to provide immune stimulation or to treat pain and inflammation which accompany the initial and fundamental HIV infection.

Thus, the methods of treatment and pharmaceutical compositions of the present invention may employ the compounds of Formula (I) in the form of monotherapy, but said methods and compositions may also be used in the form of multiple therapy in which one or more compounds of Formula (I) are coadministered in combination with one or more known therapeutic agents such as those described in detail further herein.

The present invention also provides methods of treatment in which said pharmaceutical compositions are administered to a patient. Such methods relate to treating or preventing a disease or condition by modulating CCR5 chemokine receptor activity and consequent or associated pathogenic processes subsequently mediated by the CCR5 receptor and the active ligands with which it interacts or is bound. CCR5 and the other chemotactic cytokine, *i.e.*, chemokine, receptors, play a key role in the control of a number of processes which take place in the bodies of animals. Chemokine receptors, of which more than forty different species divided into four families are presently known to exist, are proteins having a number of structural features in common, which act through chemical signaling. In the α family of chemokines, one amino acid (X) separates the first two cysteine © residues, while in the β-chemokines the first two cysteine residues are adjacent to each other (C-C). Accordingly, these two families are identified as CXC and CC chemokines, respectively. The chemokines bind specific cell-surface receptors belonging to the family of G-protein-coupled seven-transmembrane-domain proteins called "chemokine receptors", named in accordance with the class of chemokines which they bind, followed by "R" and a number. Thus, "CCR5" is a C-C chemokine receptor. See Horuk, *Trends Pharm. Sci.*, **15**,159-165 (1994) for further details. CCR5 thus belongs to the β-chemokine receptor family, which is currently known to contain eight members, CCR1 through CCR8.

The CC type of chemokine receptor interacts with various signaling proteins, including the monocyte chemoattractant proteins, MCP-1, -2, -3, -4, and -5; eotaxin-1; macrophage inflammatory proteins MIP-1α, and MIP-1β; and those regulated upon activation which are

normal T-cell expressed and secreted, RANTES. The CCR5 type of chemokine receptor in particular is known to interact with MIP-1α, MIP-1β; and RANTES in monocytes, activated T cells, dendritic cells, and natural killer cells. These β-chemokines do not act on neutrophils but rather attract monocytes, eosinophils, basophils, and lymphocytes with varying degrees of selectivity.

The present invention relates to compounds of Formula (I) which are useful in treating or preventing HIV infection, and to methods of treatment and pharmaceutical compositions containing such compounds as the active ingredient. It will be understood that the term "HIV" as used herein refers to human immunodeficiency virus (HIV), which is the etiological agent of AIDS (acquired immune deficiency syndrome), a disease that results in progressive destruction of the immune system and degeneration of the central and peripheral nervous system. Several HIV replication inhibitors are currently used as therapeutic or prophylactic agents against AIDS, and numerous others are presently under investigation.

In addition to cell-surface CD4, it has recently been shown that for entry into target cells, human immunodeficiency viruses require a chemokine receptor, CCR5 and CXCR-4 among others, as well as the virus's primary receptor CD4. The principal cofactor for entry mediated by the envelope glycoproteins of primary macrophage-tropic strains of HIV-1 is CCR5, which as already mentioned, is a receptor for the β-chemokines RANTES, MIP-1α and MIP-1β. See Deng, *et al.*, *Nature*, **381**, 661-666 (1996) for a further description of CCR5 mediated HIV entry.

HIV attaches to the CD4 molecule on cells through a region of its envelope protein, gp120, and gp120 is part of a multi-subunit complex, most likely a trimer of gp160, *i.e.*, gp120 + gp41. It is believed that the CD4 binding site on the gp120 of HIV interacts with the CD4 molecule on the cell surface, triggering conformational changes across the trimer, which allow it to bind to another cell-surface receptor, such as CCR5. This in turn enables gp41 to induce fusion with the cell membrane, and entry of the viral core into the cell. In addition, macrophage-tropic HIV and SIV envelope proteins have been shown to induce a signal through CCR5 on CD4+ cells, which may enhance the replication of the virus. See Weissman, *et al.*, *Nature*, **389**, 981-985 (1997) for a description of this phenomenon. Further, it has been shown that a complex of gp120 and soluble CD4 interacts specifically with CCR5 and inhibits the binding of the natural CCR5 ligands, as described in Wu, *et al.*, *Nature*, **384**, 179-183 (1996); and Trkola, *et al.*, *Nature*, **384**, 184-187 (1996). It has further been demonstrated that β-chemokines and related molecules, *e.g.*, (AOP)-RANTES, prevent HIV fusion to the cell membrane and subsequent infection, both *in vitro*, as described in Dragic, *et al.*, *Nature*, **381**, 667-673 (1996), and in animal models. Finally, absence of CCR5 appears to confer protection from HIV-1 infection, as described in *Nature*, **382**, 668-669 (1996). In

particular, an inherited frame-shifting mutation in the CCR5 gene, Δ32, has been shown to abolish functional expression of the gene *in vitro*, and individuals homozygous for the mutation are apparently not susceptible to HIV infection, while at the same time they do not seem to be immuno-compromised by this variant. Furthermore, those heterozygote individuals that have been infected by HIV progress more slowly to full-blown clinical AIDS. In addition to validating the role of CCR5 in the infectious cycle of HIV, the above observations suggest that CCR5 is dispensable in the adult organism.

Although most HIV-1 isolates studied to date utilize CCR5 or CXCR-4, at least nine other chemokine receptors, or structurally related molecules, have also been described as supporting HIV-1 env-mediated membrane fusion or viral entry *in vitro*. These include CCR2b, CCR3, BOB/GPR15, Bonzo/STRL33/TYMSTR, GPR1, CCR8, US28, V28/CX3CR1, LTB-4, and APJ. There is good evidence that CCR3 can be used efficiently by a significant fraction of HIV-1 isolates *in vitro*, provided that this protein is over-expressed in transfected cells. Nevertheless, consistent evidence indicates that anti-HIV drugs targeted to chemokine receptors may not be compromised by this variability. Indeed, the chemokines RANTES, MIP-1α, MIP-1β, SDF-1 have been shown to suppress replication of primary HIV isolates. A derivative of RANTES, (AOP)-RANTES, is a sub-nanomolar antagonist of CCR5 function in monocytes. Monoclonal antibodies to CCR5 have been reported to block infection of cells by HIV *in vitro*. A small molecule antagonist of CXCR4, identified as AMD3100, has been reported to inhibit infection of susceptible cultures by CXCR4 dependent primary and lab-adapted HIV viruses while another small molecule called TAK 779 blocks entry of CCR5-tropic strains (Baba, *et al. PNAS*, **96** (10), 5698-5703 (1999); In addition, the majority of primary strains from early and late disease stages utilize CCR5 exclusively or in addition to other chemokine receptors, indicating that CCR5 dependent infection may play an essential role in the initiation and maintenance of productive HIV infection in a host. Accordingly, an agent which blocks CCR5 in patients including mammals, and especially humans who possess normal chemokine receptors, can reasonably be expected to prevent infection in healthy individuals and slow or halt viral progression in infected patients.

Accordingly, the present invention is directed to the compounds of Formula (I) which inhibit the entry of human immunodeficiency virus into target cells and are therefore of value in the prevention and/or treatment of infection by HIV, as well as the prevention and/or treatment of the resulting acquired immune deficiency syndrome (AIDS). Evidence can be produced which is probative of the fact that the compounds of Formula (I) described herein inhibit viral entry through selective blockade of CCR5 dependent fusion. Consequently, the present invention also relates to pharmaceutical compositions containing the compounds of Formula (I) as an active ingredient, as well as to the corresponding method of use of the compounds of

Formula (I) as stand-alone agents, or in conjunction with other agents for the prevention and treatment of infection by HIV and resulting AIDS.

The utility of the compounds of Formula (I) of the present invention as inhibitors of HIV infection may be demonstrated by any one or more methodologies known in the art, such as the HIV microculture assays described in Dimitrov *et al.*, *J. Clin. Microbiol.* **28**, 734-737 (1990)), and the pseudotyped HIV reporter assay described in Connor *et al.*, *Virology* **206** (2) 935-44 (1995). In particular, specific compounds of Formula (I) disclosed herein as preferred embodiments are shown to inhibit p24 production following replication of laboratory-adapted and primary HIV strains in primary blood lymphocytes (PBLs) and clonal cell-lines known to support replication of both CCR5 and CXCR-4 tropic viruses, *e.g.*, PM-1 and MOLT4-clone 8. It is also noted that only those viral strains known to use CCR5 are shown to be inhibited, whereas replication of CXCR-4 tropic viruses is shown to be unaffected, indicating that compounds of Formula (I) disclosed herein are able to prevent viral entry through selective blockade of CCR5 dependent fusion. Furthermore, compounds of Formula (I) are shown to inhibit entry of chimeric HIV reporter viruses pseudotyped with envelope from a CCR5 dependent strain (ADA). Finally, compounds of Formula (I) are shown to inhibit infection of primary cells by HIV isolated from infected patient blood. Further confirmation of this anti-HIV mechanism is provided by experiments outlined below.

The ability of the compounds of Formula (I) to modulate chemokine receptor activity is demonstrated by methodology known in the art, such as the assay for CCR5 binding following procedures disclosed in Combadiere *et al.*, *J. Leukoc. Biol.* **60**, 147-52 (1996); and/or intracellular calcium mobilisation assays as described by the same authors. Cell lines expressing the receptor of interest include those naturally expressing the receptor, such as PM-1, or IL-2 stimulated peripheral blood lymphocytes (PBL), or a cell engineered to express a recombinant receptor, such as CHO, 300.19, L1.2 or HEK-293. In particular, the compounds of Formula (I) disclosed herein are shown to have activity in preventing binding of all known chemokine ligands to CCR5 in the above-mentioned binding assays. In addition, the compounds of Formula (I) disclosed herein are shown to prevent intracellular calcium mobilization in response to endogenous agonists, which is consistent with their functioning as CCR5 antagonists. For the treatment of infection by HIV and the prevention and/or treatment of the resulting acquired immune deficiency syndrome (AIDS), compounds of Formula (I) which are shown to be antagonists are preferred to compounds of Formula (I) which are shown to be agonists.

The present invention in one of its preferred embodiments is directed to the use of the compounds of Formula (I) disclosed herein for the prevention or treatment of infection by a retrovirus, in particular, the human immunodeficiency virus (HIV) and the treatment and/or

delaying of the onset of consequent pathological conditions, including but no limited to AIDS. The expressions "treating or preventing AIDS", and "preventing or treating infection by HIV" as used herein are intended to mean the treatment of a wide range of states of HIV infection: AIDS, ARC (AIDS related complex), both symptomatic and asymptomatic, and actual or potential exposure to HIV. The quoted expressions are not intended, however, to be limited to the recited treatments, but rather are contemplated to include all beneficial uses relating to conditions attributable to an AIDS causative agent. For example, the compounds of Formula (I) are useful in treating infection by HIV after suspected past exposure to HIV by, *e.g.*, blood transfusion, organ transplant, exchange of body fluids, sexual intercourse, bites, needle stick, or exposure to patient blood. In addition, a compound of Formula (I) may be used for the prevention of infection by HIV and the prevention of AIDS, such as in pre-or post-coital prophylaxis or in the prevention of maternal transmission of the HIV virus to a fetus or a child, whether at the time of birth, during the period of nursing, or in any other manner as above-described.

In a preferred embodiment of the present invention, a compound of Formula (I) may be used in a method of inhibiting the binding of human immunodeficiency virus to a chemokine receptor such as CCR5, which comprises contacting the target cell with a therapeutically effective amount of a compound of Formula (I) which is effective to inhibit the binding of the virus to the chemokine receptor. The subject treated by these preferred methods of the present invention is a mammal, preferably a human, male or female, in whom modulation of chemokine receptor activity is desired and contemplated to be efficacious. As already pointed out, the term "modulation" as used herein is intended to encompass preferably antagonism, but also agonism, partial antagonism and/or partial agonism. Also, the expression "therapeutically effective amount" as used herein is intended to mean the amount of a compound of Formula (I) as disclosed herein that will elicit the biological or medical response of a tissue, system, or animal, especially human that is being sought.

In another preferred embodiment of the present invention, a compound of Formula (I) may be used to evaluate putative retrovirus, especially HIV, mutants considered to be resistant to anti-HIV therapeutic agents, including the compounds of Formula (I) disclosed herein. Mutant viruses may be isolated from *in vitro* cultures by methods known in the art, but may also be isolated from *in vivo* animal infection models which have been disclosed in the art. More significantly, mutant viruses may be isolated from samples of patients undergoing treatment, whether optimal or sub-optimal, comprising administration of a compound of Formula (I), or any combination thereof with other known or to-be-discovered therapeutic agents. Such mutant viruses or their components, particularly their envelope proteins, may be used for several advantageous purposes, including but not limited to the following: (*i*) the evaluation and/or development of novel chemokine modulators or other agents having

improved activity against such mutant viruses; and (*ii*) the development of diagnostics capable of assisting physicians or other clinicians in the choice of a therapeutic regimen and/or outcome prediction for a patient.

In a further preferred embodiment of the present invention, compounds of Formula (I) disclosed herein are used as tools for determining the co-receptor affinity of retroviruses including HIV and SIV, or their components, especially their envelope proteins. This affinity data can be used for several advantageous purposes, including but not limited to phenotyping a given viral population, *e.g.* prior to administration of anti-retroviral therapy. The affinity data may also be used to predict the progression and outcome of the infection by the virus population involved.

In another preferred embodiment of the present invention, a compound of Formula (I) is used in the preparation and execution of screening assays for compounds which modulate the activity of chemokine, especially CCR5 receptors. For example, compounds of Formula (I) as disclosed herein are useful for isolating receptor mutants, which can then be made into screening tools for the discovery of even more potent compounds, following procedures well known in the art. Furthermore, the compounds of Formula (I) are useful in establishing or characterizing the binding sites of other ligands, including compounds other than those of Formula (I) and viral envelope proteins, to chemokine receptors, *e.g.*, by competitive inhibition. The compounds of Formula (I) are also useful for the evaluation of putative specific modulators of various chemokine receptors. As will be appreciated by the artisan, thorough evaluation of specific agonists and antagonists of the above-described chemokine receptors has been hampered by the lack of non-peptidyl, *i.e.*, metabolically resistant compounds with high binding affinity for these receptors. Thus, the compounds of Formula (I) are useful as products which may be commercially exploited for these and other beneficial purposes.

Included within the scope of the present invention are combinations of the compounds of Formula (I) with one or more therapeutic agents useful in the prevention or treatment of AIDS. For example, the compounds of the present invention may be effectively administered, whether at periods of pre-exposure and/or post-exposure to HIV, in combination with therapeutically effective amounts of known AIDS antivirals, immunomodulators, anti-infectives, or vaccines familiar to those skilled in the art. It will be understood that the scope of such combinations which include the compounds of Formula (I) is not limited to the above-recited list, but includes as well any combination with another pharmaceutically active agent which is useful for the prevention or treatment of HIV and AIDS.

Preferred combinations of the present invention include simultaneous, or sequential treatments with a compound of Formula (I) and one or more inhibitors of HIV protease and/or inhibitors of HIV reverse transcriptase, preferably selected from the class of non-nucleoside

reverse transcriptase inhibitors (NNRTI), including but not limited to nevirapine, delavirdine, and efavirenz; from among the nucleoside/nucleotide inhibitors, including but not limited to zidovudine, didanosine, zalcitabine, stavudine, lamivudine, abacavir, and adefovir dipivoxil; and from among the protease inhibitors, including but not limited to indinavir, ritonavir, saquinavir, nelfinavir, and amprenavir. Other agents useful in the above-described preferred embodiment combinations of the present invention include current and to-be-discovered investigational drugs from any of the above classes of inhibitors, including but not limited to FTC, PMPA, fozivudine tidoxil, talviraline, S-1153, MKC-442, MSC-204, MSH-372, DMP450, PNU-140690, ABT-378, and KNI-764. There is also included within the scope of the preferred embodiments of the present invention, combinations of a compound of Formula (I) together with a supplementary therapeutic agent used for the purpose of auxiliary treatment, wherein said supplementary therapeutic agent comprises one or more members independently selected from the group consisting of proliferation inhibitors, *e.g.*, hydroxyurea; immunomodulators, *e.g.*, sargramostim, and various forms of interferon or interferon derivatives; fusion inhibitors, *e.g.*, AMD3100, T-20, PRO-542, AD-349, BB-10010 and other chemokine receptor agonists/antagonists; integrase inhibitors, *e.g.*, AR177; RNaseH inhibitors; inhibitors of viral transcription and RNA replication; and other agents that inhibit viral infection or improve the condition or outcome of HIV-infected individuals through different mechanisms.

Preferred methods of treatment of the present invention for the prevention of HIV infection, or treatment of aviremic and asymptomatic subjects potentially or effectively infected with HIV, include but are not limited to administration of a member independently selected from the group consisting of: (*i*) a compound within the scope of Formula (I) as disclosed herein; (*ii*) one NNRTI in addition to a compound of (*i*); (*iii*) two NRTI in addition to a compound of (*i*); (*iv*) one NRTI in addition to the combination of (*ii*); and (*v*) a compound selected from the class of protease inhibitors used in place of an NRTI in combinations (*iii*) and (*iv*).

The preferred methods of the present invention for therapy of HIV-infected individuals with detectable viremia or abnormally low CD4 counts further include as a member to be selected: (*vi*) treatment according to (*i*) above in addition to the standard recommended initial regimens for the therapy of established HIV infections, *e.g.*, as described in Bartlett, J. G., "1998 Medical management of HIV infection", Johns Hopkins University publishers, ISBN 0-9244-2809-0. Such standard regimens include but are not limited to an agent from the class of protease inhibitors in combination with two NRTIs; and (*vii*) a standard recommended initial regimens for the therapy of established HIV infections, *e.g.*, as described in Bartlett, J. G., "1998 Medical management of HIV infection", Johns Hopkins University publishers, ISBN 0-

9244-2809-0), where either the protease inhibitor component, or one or both of the NRTIs is/are replaced by a compound within the scope of Formula (I) as disclosed herein.

The preferred methods of the present invention for therapy of HIV-infected individuals that have failed antiviral therapy further include as a member to be selected: (*viii*) treatment according to (*i*) above, in addition to the standard recommended regimens for the therapy of such patients, *e.g.*, as described in Bartlett, J. G., "1998 Medical management of HIV infection", Johns Hopkins University publishers, ISBN 0-9244-2809-0); and (*ix*) a standard recommended initial regimens for the therapy of patients who have failed antiretroviral therapy, *e.g.*, as described in Bartlett, J. G., "1998 Medical management of HIV infection", Johns Hopkins University publishers, ISBN 0-9244-2809-0), where either one of the protease inhibitor components, or one or both of the NRTIs is/are replaced by a compound within the scope of Formula (I) as disclosed herein.

In the above-described preferred embodiment combinations of the present invention, the compound of Formula (I) and other therapeutic active agents may be administered in terms of dosage forms either separately or in conjunction with each other, and in terms of their time of administration, either serially or simultaneously. Thus, the administration of one component agent may be prior to, concurrent with, or subsequent to the administration of the other component agent(s).

The compounds of Formula (I) may be administered in accordance with a regimen of 1 to 4 times per day, preferably once or twice per day. The specific dose level and frequency of dosage for any particular patient may be varied and will depend upon a variety of factors including the activity of the specific compound employed, the metabolic stability and length of action of that compound, the age, body weight, general health, sex, diet, mode and time of administration, rate of excretion, drug combination, the severity of the particular condition, and the host undergoing therapy. In particular, however, the treatment of retroviral infections, and more particularly HIV, may be guided by genotyping and phenotyping the virus in the course of or prior to the initiation of administration of the therapeutic agent. In this way, it is possible to optimise dosing regimens and efficacy when administering a compound of Formula (I) for the prevention or treatment of infection by a retrovirus, in particular, the human immunodeficiency virus (HIV).

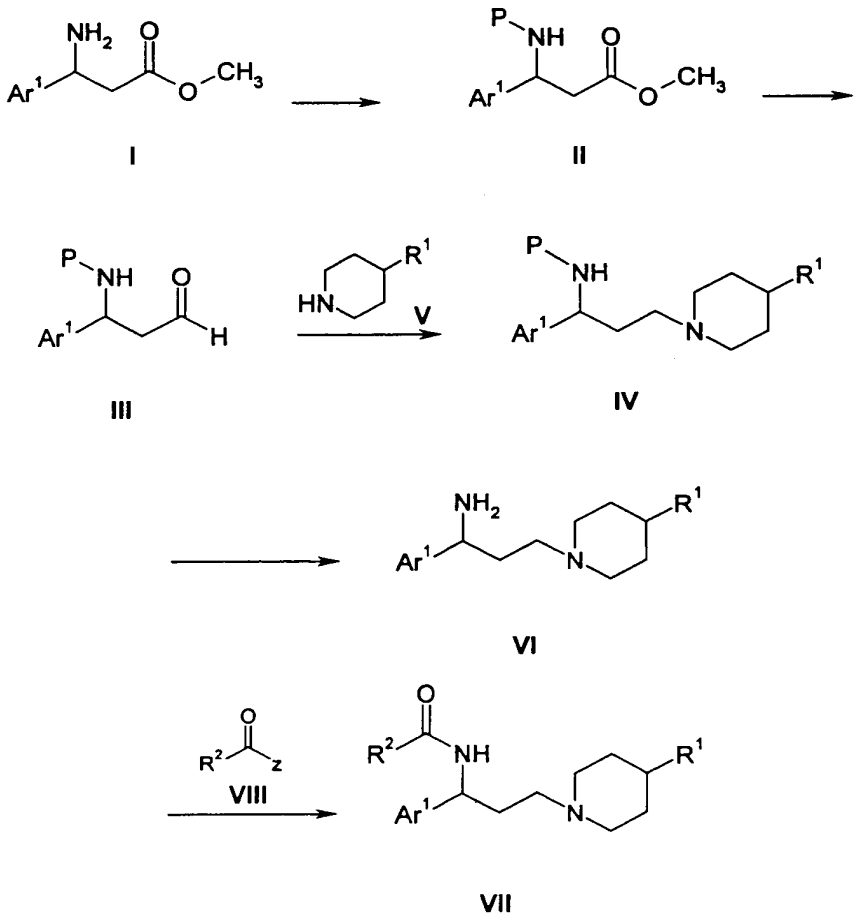
The compounds of this invention may be used for treatment of respiratory disorders, including: adult respiratory distress syndrome (ARDS), bronchitis, chronic bronchitis, chronic obstructive pulmonary disease, cystic fibrosis, asthma, emphysema, rhinitis and chronic sinusitis.

The invention is further described by means of examples, but not in any limitative sense.

The following general synthetic routes may be employed.

METHODS OF PREPARING COMPOUNDS OF THE PRESENT INVENTION

Synthesis I



Preparation of the compounds of formula **II** from the amino acid derivative **I** where P is a suitable protecting group (preferably BOC), may be achieved for example, by reaction with di-tert-butyl dicarbonate in the presence of a base such as aqueous sodium hydroxide in a suitable solvent such as tetrahydrofuran.

Compounds of formula **III** may be prepared by reduction of compounds of formula **II**, using a suitable reducing agent, preferably diisobutylaluminium hydride in dichloromethane at -78°C.

Compounds of the general formula **IV** may be prepared by the reductive alkylation of an appropriate amine of formula **V**, with an aldehyde, of formula **III**. The reaction may be carried out in the presence of an excess of suitable reducing agent (e.g. sodium triacetoxyborohydride) in a protic solvent system (acetic acid in dichloromethane or 1,1,1-trichloroethane), at room temperature.

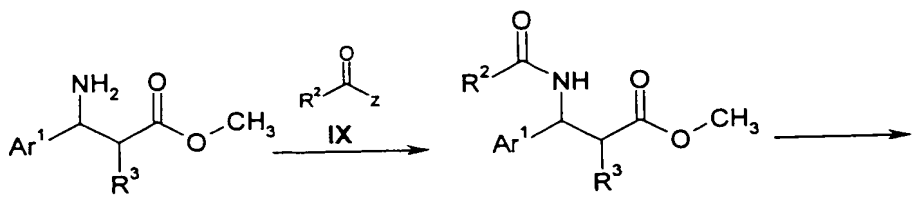
Subsequent removal of the nitrogen protecting group may be achieved using trifluoroacetic acid or hydrochloric acid in a solvent such as dioxane or dichloromethane at room temperature for from 1 to 60 hours to provide the compound of formula **VI**. Compounds of general formula **VII** may be prepared by coupling the amine of formula **VI** with an acid (Z = OH) or acid derivative (e.g., Z = Cl) of formula **VIII** using conventional amide bond forming techniques. For example, the acid **VIII** may be activated using a carbodiimide such as 3-(3-dimethylamino-1-propyl)-1-ethylcarbodiimide, optionally in the presence of 1-hydroxybenzotriazole hydrate. These reactions may be performed in a suitable solvent such as dichloromethane, optionally in the presence of a tertiary amine, such as triethylamine or N-ethyldiisopropylamine at about room temperature.

Alternatively an acyl chloride of formula **VIII**, may be reacted with an amine of formula **VI** in the presence of a tertiary amine, such as triethylamine or N-diisopropylethylamine in a suitable solvent such as dichloromethane at room temperature.

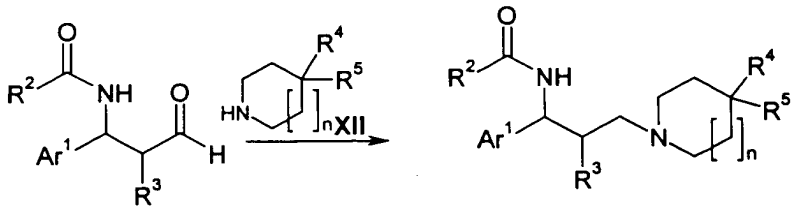
In a further variation a compound of formula **VII**, may be formed in a "one-pot procedure" by deprotection of a compound of formula **IV**, and coupling the resultant amine of formula **VI** with the acid derivative of formula **VIII**, using methods previously described.

When a compound of formula **I** is required as a single enantiomer it may be obtained according to the method of Davies et al. (J. Chem. Soc. Perk. Trans. I; 9; 1994; 1129).

Synthesis II



VIII **X**



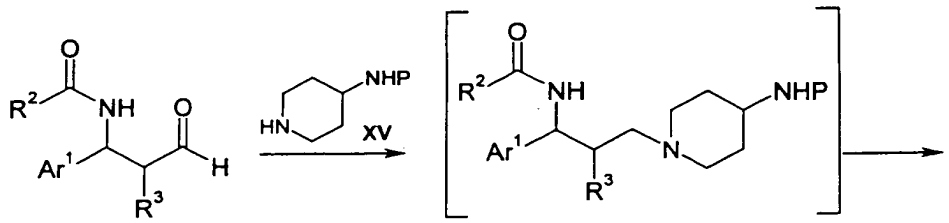
XI XIII

n = 0 or 1

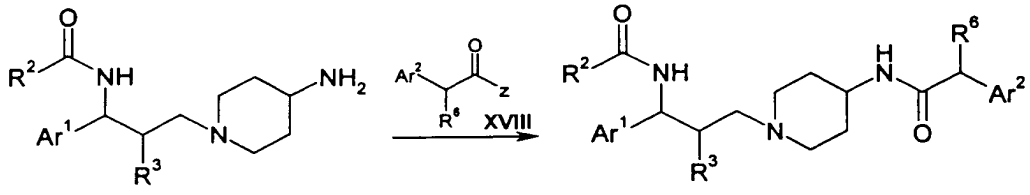
Compounds of formula **X** may be prepared by coupling the amino acid derivative of formula **VIII** with an acid (Z = OH) or acid derivative (e.g., Z = Cl) of formula **IX** using conventional amide bond forming techniques as described in synthesis I. R^3 may be hydrogen or OH. In the case of R^3 = OH methylation to R^3 = OCH_3 may be performed using, for example, iodomethane and silver oxide in acetonitrile at reflux. Compounds of formula **XI** may be prepared by reduction of compounds of formula **X**, according to the method described in synthesis I. Reductive alkylation of the amine of formula **XII**, with the aldehyde of formula **XI**, according to the method described in synthesis I, may provide the compounds of formula **XIII**.

When a compound of formula **VIII** is required as a single enantiomer it may be obtained according to the method of Davies et al. (J. Chem. Soc. Perk. Trans. I; 9; 1994; 1129).

Synthesis III



XI



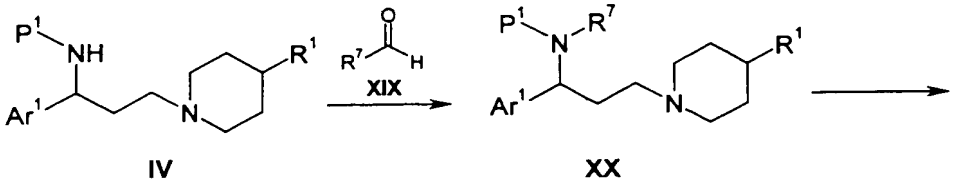
XVI XVII

Compounds of the general formula **XVI** may be prepared by the reductive alkylation of an appropriate amine of formula **XV**, where P is a suitable protecting group (preferably trifluoroacetyl), with an aldehyde of formula **XI**. The reaction may be carried out in the presence of an excess of suitable reducing agent (e.g. sodium triacetoxyborohydride) in a protic solvent system (acetic acid in 1,1,1-trichloroethane), at room temperature.

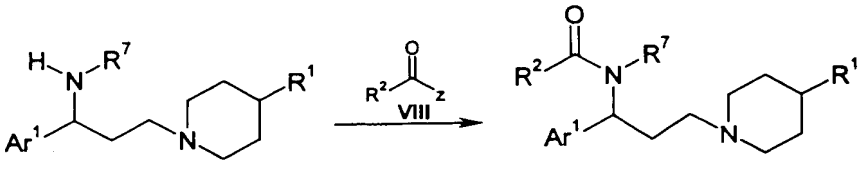
Subsequent removal of the nitrogen protecting group in a "one-pot procedure" may be achieved using, for example, an excess of aqueous sodium hydroxide in a solvent such as ethanol at room temperature for 1 hour to provide the compound of formula **XVI**.

Compounds of formula **XVII** may be prepared by coupling the amine of formula **XVI** with an acid (Z = OH) or acid derivative (e.g., Z = Cl) of formula **XVIII** using conventional amide bond forming techniques as described in synthesis I.

Synthesis IV



IV **XX**



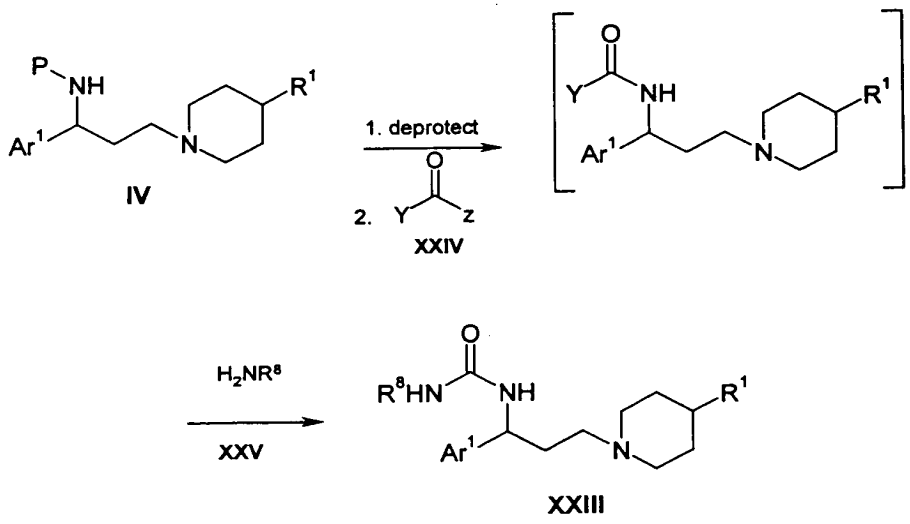
XXI **XXII**

Compounds of the formula **XX** (R^7 is preferably lower alkyl, for example methyl) may be prepared by reacting the protected amine (where P^1 is preferably benzyl) of formula **IV**, and the aldehyde of formula **XIX**. Typically, the reaction may be carried out with formaldehyde in formic acid and stirring the reaction mixture at 100°C for 1 hour following the Eschweiler-Clarke procedure.

Subsequent removal of the nitrogen protecting group may be achieved under conditions of transfer hydrogenation, using a catalyst such as Pearlman's catalyst in the presence of excess ammonium formate in a suitable solvent conditions such as ethanol at reflux to provide the compound of formula **XXI**.

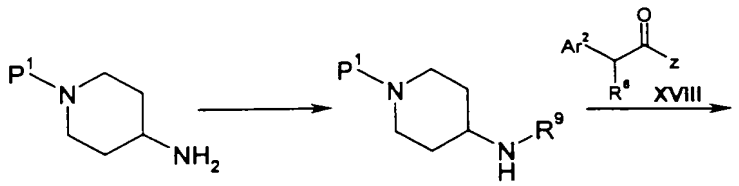
Compounds of formula **XXII** may be prepared by coupling the amine derivative of formula **XXI** with an acid (Z = OH) or acid derivative (e.g., Z = Cl) of formula **VIII** using conventional amide bond forming techniques as described in synthesis I.

Synthesis V

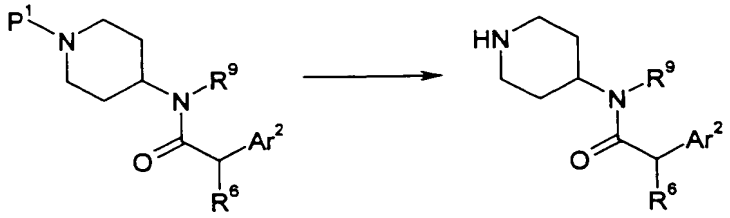


Compounds of the general formula **XXIII** may be prepared from the amine of formula **IV** by removal of the nitrogen protecting group as described in synthesis 1 and subsequent coupling with an acyl derivative (preferably Y = Z = imidazolyl), using aprotic solvents such as dichloromethane, and base, preferably imidazole, between 0°C and room temperature over 1 hour. The intermediate may then be treated at room temperature with the amine of formula **XXV**, for example pyrrolidine, to yield the urea of the general structure **XXIII**.

Synthesis VI



XXVI XXVII XVIII



XXVIII XXIX

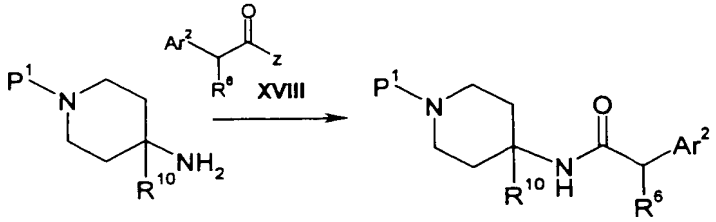
The preparations of the intermediates of the general formula **V**, for example compounds of substructures such as **XXIX** and **XXXII**, are described in more detail in syntheses **VI** and **VII**.

The amine of formula **XXVII** may be prepared from the compound of formula **XXVI**, where P1 is a suitable protecting group (preferably benzyl), by treatment with a carbamoyl chloride, for example benzyl chloroformate, at about 0°C and subsequent reduction of the intermediate, using a suitable reducing agent, such as lithium aluminium hydride at reflux for 12 hours in a suitable solvent such as ether or tetrahydrofuran.

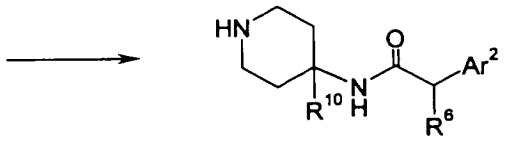
Compounds of formula **XXVIII** may be prepared by coupling the amine derivative of formula **XXVII** with an acid (Z = OH) or acid derivative (e.g., Z = Cl) of formula **XVIII** using conventional amide bond forming techniques as described in synthesis I.

Subsequent removal of the nitrogen protecting group may be achieved under conditions of transfer hydrogenation, using a catalyst such as Pearlman's catalyst in the presence of excess ammonium formate in a suitable solvent conditions such as ethanol at reflux to provide the compound of formula **XXIX**.

Synthesis VII



XXX XXXI XVIII



XXXII

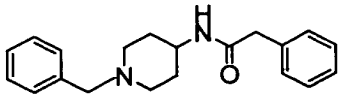
The amide derivative of formula **XXXI** may be prepared from compound **XXX**, where P^1 is a suitable protecting group (preferably benzyl), by coupling with an acid (Z = OH) or acid derivative (e.g., Z = Cl) of formula **XVIII** using conventional amide bond forming techniques as described in synthesis I.

Subsequent removal of the nitrogen protecting group may be achieved under conditions of transfer hydrogenation, using a catalyst such as Pearlman's catalyst in the presence of excess ammonium formate in a suitable solvent conditions such as ethanol at reflux to provide the compound of formula **XXXII**.

EXPERIMENTAL SECTION

PREPARATION 1

N-(1-Benzyl-4-piperidinyl)-2-phenylacetamide

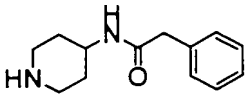


Phenacetyl chloride (7.29ml, 55.2mmol) was added dropwise to a diethyl ether (100ml) solution of 1-benzyl-4-piperidinamine (10.0g, 52.6mmol) and triethylamine (1.60ml, 11.5mmol) at 0°C. The reaction mixture was stirred for 30 minutes. The solution was washed with saturated sodium bicarbonate solution, water and brine, then dried ($MgSO_4$), filtered and the solvent removed under reduced pressure to afford the title compound as a white solid, 16.2g.

^{1}H NMR (300MHz, $CDCl_3$): δ [ppm] 1.23-1.39 (2H, m), 1.79-1.90 (2H, m), 2.08 (2H, t), 2.68-2.77 (2H, m), 3.45 (2H, s), 3.55 (2H, s), 3.73-3.87 (1H, m), 5.13-5.24 (1H, m), 7.20-7.39 (10H, m).

PREPARATION 2

2-Phenyl-*N*-(4-piperidinyl)acetamide

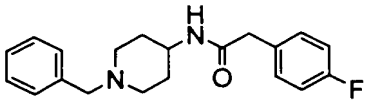


To a solution of the title compound of preparation 1 (16g, 52mmol) in ethanol (100 ml) was added palladium hydroxide (5g), followed by portionwise addition of ammonium formate (16.3g, 259mmol). The reaction was heated to reflux for 30 minutes, then cooled and filtered through a pad of Arbocel®. The solvent was removed under reduced pressure to yield the title product as white foam, 8g.

^{1}H NMR (300MHz, $CDCl_3$): δ [ppm] 1.11-1.27 (2H, m), 1.81-1.90 (2H, m), 2.08 (1H, s), 2.65 (2H, t), 2.94-3.03 (2H, m), 3.55 (2H, s), 3.80-3.94 (1H, m), 5.19-5.32 (1H, m), 7.26-7.40 (5H, m).

PREPARATION 3

N-(1-Benzyl-4-piperidinyl)-2-(4-fluorophenyl)acetamide

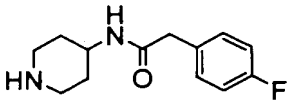


1-Benzyl-4-piperidinylamine (5g, 26.1mmol), 2-(4-fluorophenyl)acetic acid (4g, 26mmol), 1-hydroxybenzotriazole monohydrate (3.89g, 28.6mmol), 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (6g, 31.2mmol) and triethylamine (4.4ml, 31.2mmol) were stirred together for 18 hours in dichloromethane (250ml). The mixture was evaporated under reduced pressure and the residue dissolved in ethyl acetate before washing with water (x3). The organic layer was dried ($MgSO_4$), filtered and evaporated under reduced pressure to afford the title compound as a white solid, 9g.

^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] 1.24-1.40 (2H, m), 1.77-1.87 (2H, m), 2.00-2.11 (2H, m), 2.65-2.74 (2H, m), 3.47 (2H, s), 3.48 (2H, s), 3.71-3.82 (1H, m), 5.13-5.23 (1H, m), 6.97-7.06 (2H, m), 7.16-7.32 (7H, m).

PREPARATION 4

2-(4-Fluorophenyl)-*N*-(4-piperidinyl)acetamide

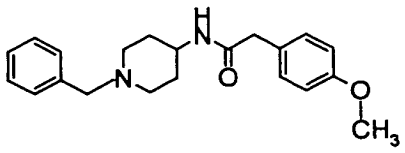


The title compound of preparation 3 (2.85g, 8.73mmol), ammonium formate (2.85g, 45.2mmol) and 20% palladium hydroxide on carbon (600mg) were heated in ethanol (50ml) at reflux until gas evolution ceased. After cooling to room temperature the mixture was filtered through Arbocel® and the filtrate evaporated under reduced pressure to afford the title compound as a gum, 2.2g.

^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] 1.26-1.40 (2H, m), 1.84-1.94 (2H, m), 2.65-2.74 (2H, m), 3.03-3.11 (2H, m), 3.50 (2H, s), 3.82-3.94 (1H, m), 5.40-5.48 (1H, m), 6.98-7.05 (2H, m), 7.18-7.24 (2H, m).

PREPARATION 5

N-(1-Benzyl-4-piperidinyl)-2-(4-methoxyphenyl)acetamide



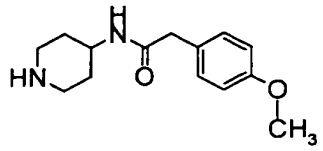
1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (2.42g, 12.6mmol) was added to a solution of 1-benzyl-4-piperidinamine (2.0g, 10.5mmol), 4-methoxyphenylacetic acid (1.83g, 11.0mmol), 1-hydroxybenzotriazole hydrate (1.56g, 11.5mmol) and triethylamine (2.70ml, 26.2mmol) in dichloromethane (100ml). The reaction mixture was stirred for 18 hours after which time the solvent was evaporated under reduced pressure. The residue was dissolved in ethyl acetate and washed with water (3x), dried ($MgSO_4$), filtered and the solvent removed under reduced pressure to afford the title compound as a white solid, 3.44g.

^{1}H-NMR (300MHz, $CDCl_3$) : δ [ppm] 1.22-1.40 (2H, m), 1.81 (2H, d), 2.09 (2H, t), 2.70 (2H, d), 3.45 (2H, s), 3.50 (2H, s), 3.72-3.87 (4H, m), 5.20 (1H, d), 6.88 (2H, d), 7.15 (2H, d), 7.26 (5H, m).

LRMS : m/z 339 (MH^+)

PREPARATION 6

2-(4-Methoxyphenyl)-*N*-(4-piperidinyl)acetamide

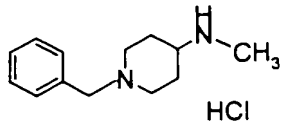


Obtained from the title compound of preparation 5 as a clear oil in 92% yield using a similar procedure to that in preparation 2.

^{1}H-NMR (300MHz, $CDCl_3$) : δ [ppm] 1.32-1.68 (2H, m), 1.88 (2H, d), 2.36 (2H, s), 2.50 (2H, t), 2.96-3.05 (2H, m), 3.46 (2H, s), 3.79-3.92 (3H, m), 5.27 (1H, d), 6.88 (2H, d), 7.15 (2H, d).

PREPARATION 7

1-Benzyl-*N*-methyl-4-piperidinamine hydrochloride



HCl

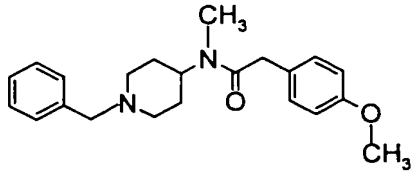
Benzyl chloroformate (4.44g, 26.0mmol) in ether (10ml) was added dropwise to an ether (50ml) solution of *N*-benzyl-4-piperidinamine (4.50g, 23.6mmol) and aqueous sodium hydrogen carbonate (50ml) at 0°C. After addition was complete the reaction was warmed to room temperaure and the layers separated, the organic layer was washed with brine, dried ($MgSO_4$), filtered and the solvent removed under reduced pressure. The residue was dissolved in tetrahydrofuran (80ml) and lithium aluminium hydride (2.69g, 70.8mmol) was added portionwise and the reaction heated under reflux for 12 hours. Upon cooling to 0°C water (4ml) was cautiously added followed by 2N sodium hydroxide (4ml) and the precipitate removed by filtration. The solvent was removed under reduced pressure to yield a yellow oil which was dissolved in ether (100ml) and hydrogen chloride gas was bubbled through to give a white precipitate. Filtration furnished the title compound as a white solid, 4.30g.

^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] 2.04 (2H, m), 2.38 (2H, d), 2.78 (3H, s), 3.18 (2H, t), 3.43 (1H, t), 3.60 (2H, d), 4.38 (2H, s), 7.45 (3H, m), 7.58 (2H, m).

LRMS: m/z 205 (MH^+)

PREPARATION 8

N-(1-Benzyl-4-piperidinyl)-2-(4-methoxyphenyl)-*N*-methylacetamide



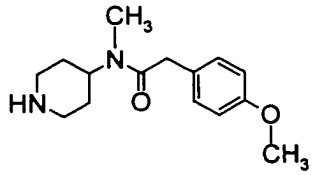
4-Methoxyphenylacetic acid (895mg, 5.39mmol), triethylamine (2.40ml, 17.1mmol) and 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (1.12g, 5.88mmol) were added to a solution of the title compound of preparation 7 (1.00g, 4.90mmol) in dichloromethane (20ml). The mixture was stirred for 12 hours at room temperature, then partitioned between dichloromethane and water. The aqueous layer was separated and extracted with dichloromethane (2x). The combined organic extracts were washed with brine, dried ($MgSO_4$), filtered and evaporated under reduced pressure. The residual yellow oil was purified by column chromatography on silica gel using ethyl acetate:methanol (90:10) as eluant to afford the title compound as a clear oil, 1.26g.

^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm] 1.28-1.38 (1H, m), 1.45-1.95 (3H, m), 2.09-2.17 (1H, m), 2.81 (3H, s), 2.83-2.98 (2H, m), 3.42-3.52 (2H, m), 3.61-3.70 (2H, m), 3.78 (3H, s), 4.42-4.59 (1H, m), 6.80-6.90 (2H, m), 7.10-7.20 (2H, m), 7.20-7.37 (5H, m).

LRMS: m/z 353 (MH^+)

PREPARATION 9

2-(4-Methoxyphenyl)-*N*-methyl-*N*-(4-piperidinyl)acetamide



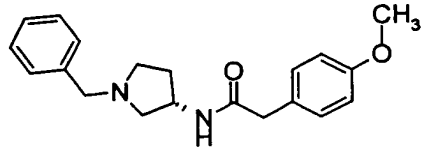
Obtained from the title compound of preparation 8 as a clear oil in 82% yield using a similar procedure to that in preparation 2.

^1H NMR (300 MHz, $CDCl_3$): δ [ppm] 1.23-1.35 (1H, m); 1.45-1.65 (3H, m); 2.40-2.58 (3H, m); 2.75 (3H, s); 2.99-3.15 (2H, m); 3.57-3.69 (2H, m); 3.73 (3H, s); 4.45-4.61 (1H, m); 6.79 (2H, d); 7.10 (2H, d).

LRMS: m/z 263 (MH^+)

PREPARATION 10

N-[(3*S*)-1-Benzylpyrrolidinyl]-2-(4-methoxyphenyl)acetamide



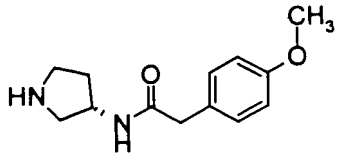
4-Methoxyphenylacetic acid (519mg, 3.13mmol), 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (651mg, 3.39mmol) and 1-hydroxybenzotriazole hydrate (458mg, 3.39mmol) were added to a solution of (3*S*)-1-benzyl-3-aminopyrrolidine (500mg, 2.83mmol) in dichloromethane (10ml). The mixture was stirred for 12 hours at room temperature, then partitioned between dichloromethane and water. The aqueous layer was separated and extracted with dichloromethane (2x). The combined organic extracts were washed with brine, dried ($MgSO_4$), filtered and evaporated under reduced pressure to give the title compound as a pale pink solid, 600mg.

^1H NMR (300MHz, $CDCl_3$) : δ [ppm] 1.43 (1H, m), 2.20 (2H, m), 2.46 (2H, m), 2.78 (1H, t), 3.47 (2H, s), 3.52 (2H, q), 3.80 (3H, s), 4.40 (1H, m), 5.78 (1H, br d), 6.87 (2H, d), 7.15-7.35 (7H, m).

LRMS : m/z 325 (MH^+)

PREPARATION 11

2-(4-Methoxyphenyl)-*N*-[(3*S*)-pyrrolidinyl]acetamide



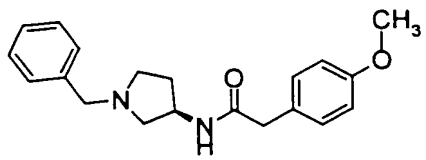
Obtained from the title compound of preparation 10 as a white solid in 98% yield using a similar procedure to that in preparation 2.

R_F = 0.23 (dichloromethane:methanol:0.88 ammonia 80:20:1)

LRMS: m/z 235 (MH^+)

PREPARATION 12

N-[(3*R*)-1-Benzylpyrrolidinyl]-2-(4-methoxyphenyl)acetamide



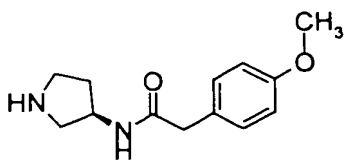
4-Methoxyphenylacetic acid (519mg, 3.13mmol), 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (651mg, 3.39mmol) and 1-hydroxybenzotriazole hydrate (458mg, 3.39mmol) were added to a solution of (3*R*)-1-benzyl-3-aminopyrrolidine (500mg, 2.83mmol) in dichloromethane (10ml). The mixture was stirred for 12 hours at room temperature, then partitioned between dichloromethane and water. The aqueous layer was separated and extracted with dichloromethane (2x). The combined organic extracts were washed with brine, dried ($MgSO_4$), filtered and evaporated under reduced pressure. The residual pink solid was purified by column chromatography on silica gel using dichloromethane:methanol:0.88 ammonia (95:5:0.5) as eluant to afford the title compound as a white solid, 760mg.

^1H NMR (400MHz, $CDCl_3$) : δ [ppm] 1.43 (1H, m), 2.20 (2H, m), 2.46 (2H, m), 2.78 (1H, t), 3.47 (2H, s), 3.52 (2H, q), 3.80 (3H, s), 4.40 (1H, m), 5.78 (1H, br d), 6.87 (2H, d), 7.15-7.35 (7H, m).

LRMS : m/z 325 (MH^+)

PREPARATION 13

2-(4-Methoxyphenyl)-*N*-[(3*R*)-pyrrolidinyl]acetamide



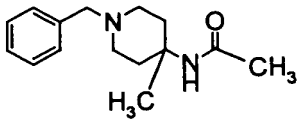
Obtained from the title compound of preparation 12 as a white solid in 92% yield using a similar procedure to that in preparation 2.

R_F = 0.23 (dichloromethane:methanol:0.88 ammonia 80:20:1)

LRMS: m/z 235 (MH^+)

PREPARATION 14

N-(1-Benzyl-4-methyl-4-piperidinyl)acetamide



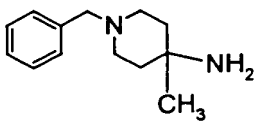
Concentrated sulphuric acid (3ml) was added dropwise to a solution of 1-benzyl-4-methyl-4-piperidinol (640mg, 2.56mmol) in acetonitrile (4ml) at 0°C. After 1 hour the reaction was cautiously poured onto 2N sodium hydroxide (20ml), the layers separated and the aqueous extracted with dichloromethane (3x). The combined organic extracts were washed with water, brine, dried (K_2CO_3), filtered and evaporated under reduced pressure to afford the title compound as a clear oil, 600mg.

^1H NMR (400 MHz, $CDCl_3$): δ [ppm] 1.38 (3H, s), 1.61-1.71 (2H, m), 1.92 (3H, s), 1.98-2.08 (2H, m), 2.18-2.24 (2H, m), 2.56-2.60 (2H, m), 3.50 (2H, s), 7.20-7.38 (5H, m).

LRMS: m/z 247 (MH^+)

PREPARATION 15

1-Benzyl-4-methyl-4-piperidinamine



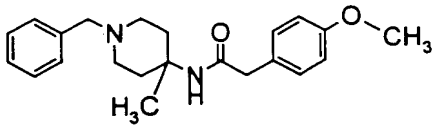
A solution of the title compound of preparation 14 (670mg, 3.28mmol) in concentrated hydrochloric acid (15ml) was heated under reflux for 24 hours and then cooled. The reaction mixture was cautiously poured onto 2N sodium hydroxide (50ml) solution and the aqueous solution extracted with dichloromethane (3x). The combined organic extracts were washed with brine, dried ($MgSO_4$), filtered and evaporated under reduced pressure to afford the title compound as a brown oil, 490mg.

^1H NMR (400 MHz, CD_3OD): δ [ppm] 1.09 (3H, s), 1.43-1.60 (4H, m), 2.38-2.53 (4H, m), 3.51 (2H, s), 7.20-7.37 (5H, m).

LRMS: m/z 204 (MH^+)

PREPARATION 16

N-(1-Benzyl-4-methyl-4-piperidinyl)-2-(4-methoxyphenyl)acetamide



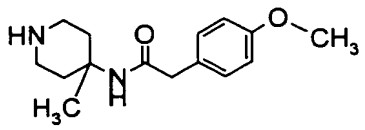
4-Methoxyphenylacetic acid (390mg, 2.36mmol) and 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (330mg, 1.73mmol) were added to a solution of the title compound of preparation 15 (320mg, 1.57mmol) in dichloromethane (10ml). The mixture was stirred for 12 hours at room temperature, then partitioned between dichloromethane and water. The aqueous layer was separated and extracted with dichloromethane (2x). The combined organic extracts were washed with brine, dried ($MgSO_4$), filtered and evaporated under reduced pressure. The residual yellow oil was purified by column chromatography on silica gel using an elution gradient of ethyl acetate:methanol:0.88 ammonia (100:0:0 to 95:5:0.5) to afford the title compound as a white solid, 270mg.

^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] 1.32 (3H, s), 1.50-1.61 (2H, m), 1.82-1.98 (4H, m), 2.43-2.52 (2H, m), 3.40 (2H, s), 3.43 (2H, s), 3.81 (3H, s), 6.90 (2H, d), 7.15 (2H, d), 7.21-7.35 (5H, m).

LRMS: m/z 353 (MH^+)

PREPARATION 17

2-(4-Methoxyphenyl)-*N*-(4-methyl-4-piperidinyl)acetamide



Obtained from the title compound of preparation 16 as a yellow oil in 94% yield using a similar procedure to that in preparation 2.

^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] 1.12 (3H, s), 1.41-1.52 (2H, m), 1.85-1.95 (2H, m), 2.35 (2H, br s), 2.48-2.60 (2H, m), 2.70-2.80 (2H, m), 3.42 (2H, s), 3.69 (3H, s), 6.83 (2H, d), 7.15 (2H, d).

LRMS: m/z 263 (MH^+)

PREPARATION 18

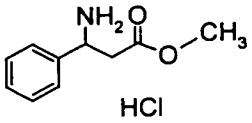
3-[(*t*-Butoxycarbonyl)amino]-3-phenylpropanoic acid

A solution of di-*t*-butyldicarbonate (17.1g, 78.7mmol) in tetrahydrofuran (60ml) was added to a solution of DL-3-amino-3-phenylpropionic acid (10.0g, 60.5mmol) in aqueous 2N sodium hydroxide solution (145ml) and the reaction stirred at room temperature for 18 hours. The reaction was diluted with water, washed with ethyl acetate (2x) and then acidified to pH 3 using concentrated hydrochloric acid. This acidic solution was extracted with ethyl acetate (2x), the combined organic extracts dried ($MgSO_4$), filtered and evaporated under reduced pressure to afford the title compound as an off-white solid, 15.3g.

^{1}H NMR (300MHz, $CDCl_3$) : δ [ppm] 1.40 (9H, s), 2.86 (2H, br s), 5.08 (1H, br s), 7.30 (5H, m).

PREPARATION 19

Methyl-3-amino-3-phenylpropanoate hydrochloride

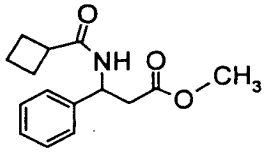


3-Phenyl-β-alanine (13.0g, 78.8mmol) was dissolved in methanolic hydrochloric acid (200ml, 2.25M). The reaction mixture was heated under reflux for 18 hours, then the cooled reaction mixture was concentrated under reduced pressure to afford the title compound as a yellow oil, 16.9g.

^{1}H-NMR (400MHz, CD_3OD) : δ [ppm] 3.00-3.19 (2H, m), 3.72 (3H, s), 4.74 (1H, t), 7.48 (5H, s).

PREPARATION 20

Methyl-3-[(cyclobutylcarbonyl)amino]-3-phenylpropanoate



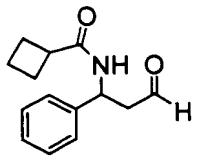
Cyclobutanecarbonyl chloride (6.91ml, 86.7mmol) was added dropwise to a solution of the title compound of preparation 19 (17.0g, 78.8mmol) and triethylamine (24.2ml, 173.4mmol) in dichloromethane (200ml) at 0°C. The reaction mixture was stirred for 56 hours at room temperature after which time the mixture was washed with water then brine, dried ($MgSO_4$), filtered and the solvent removed under reduced pressure to afford the title compound as a yellow oil, 20.8g.

^{1}H-NMR (400MHz, $CDCl_3$) : δ [ppm] 2.00-2.10 (2H, m), 2.10-2.35 (4H, m), 2.80-3.00 (2H, m), 3.03 (1H, m), 3.62 (3H, s), 5.42 (1H, m), 6.50 (1H, d), 7.25-7.35 (5H, m).

LRMS : m/z 262 (MH^+)

PREPARATION 21

N-(3-Oxo-1-phenylpropyl)cyclobutanecarboxamide



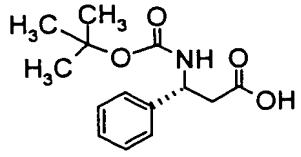
Diisobutylaluminium hydride (42.1ml of a 1.0M solution in dichloromethane, 42.1mmol) was added dropwise to a solution of the title compound of preparation 20 (5.0g, 19.1mmol) in dichloromethane (100ml) at -78°C. The reaction mixture was stirred at this temperature for a further 1 hour, then methanol (5ml) precooled to -78°C was added. The mixture was warmed to room temperature and washed with 2N hydrochloric acid, water, brine, dried ($MgSO_4$), filtered and the solvent evaporated under reduced pressure to afford the title compound as a yellow oil, 3.3g.

^{1}H-NMR (400MHz, $CDCl_3$) : δ [ppm] 1.81-2.35 (6H, m), 2.90-3.10 (3H, m), 5.50 (1H, m), 6.00 (1H, br d), 7.23-7.39 (5H, m), 9.75 (1H, m).

LRMS : m/z 232 (MH^+)

PREPARATION 22

(3*R*)-3-[(*t*-Butoxycarbonyl)amino]-3-phenylpropanoic acid

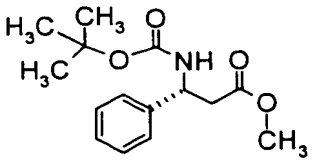


Obtained from (3*R*)-3-amino-3-phenylpropanoic acid as a white solid in 98% yield using a similar procedure to that in preparation 18.

^{1}H-NMR (400MHz, $CDCl_3$) : δ [ppm] 1.43 (9H, br s), 2.83 (2H, br m), 5.12 (1H, br m), 5.40 (1H, br m), 7.36 (5H, m).

PREPARATION 23

Methyl (3*R*)-3-[(*t*-butoxycarbonyl)amino]-3-phenylpropanoate



A mixture of the title compound of preparation 22 (3.1g, 11.7mmol), 4-dimethylaminopyridine (0.11g, 0.90mmol), N,N'-dicyclohexylcarbodiimide (2.5g, 12.1mmol)

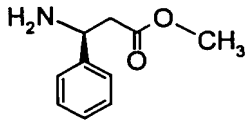
and dry methanol (1.8ml, 44mmol) in dichloromethane (80ml) was stirred at room temperature for 18 hours. The mixture was filtered, the filtrate evaporated under reduced pressure and the solid re-suspended in ether. This suspension was filtered, and the filtrate evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel, using an elution gradient of dichloromethane:methanol (99:1 to 95:5) to afford the title compound, 3.2g.

^{1}H-NMR (400MHz, $CDCl_3$) : δ [ppm] 1.42 (9H, br s), 2.83 (2H, br m), 3.62 (3H, s), 5.12 (1H, br m), 5.42 (1H, br m), 7.28 (5H, m).

LRMS : m/z 280 (MH^+)

PREPARATION 24

Methyl-(3*S*)-3-amino-3-phenylpropanoate

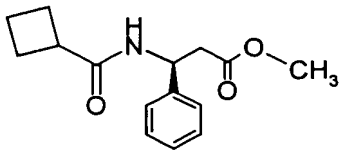


t-Butyl (3*S*)-3-amino-3-phenylpropanoate (1.0g, 4.97mmol) was dissolved in methanolic hydrochloric acid (2.25M, 25ml). The reaction mixture was heated under reflux for 3 hours, after which time the solvent was evaporated under reduced pressure and the residue basified with aqueous saturated sodium carbonate solution. The product was then extracted using dichloromethane (x2), the combined organic layers were dried ($MgSO_4$), filtered and the solvent removed under reduced pressure to afford the title compound as a white solid, 778mg.

^{1}H-NMR (400MHz, $CDCl_3$) : δ [ppm] 1.70 (2H, s), 2.66 (2H, d), 3.68 (3H, s), 4.43 (1H, t), 7.25-7.40 (5H, m).

PREPARATION 25

Methyl-(3*S*)-3-[(cyclobutylcarbonyl)amino]-3-phenylpropanoate

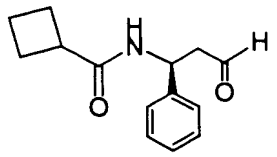


Obtained from the title compound of preparation 24 as a brown solid in 82% yield using a similar procedure to that in preparation 20.

^{1}H-NMR (300MHz, $CDCl_3$) : δ [ppm] 1.81-2.06 (2H, m), 2.10-2.40 (5H, m), 2.82-3.08 (2H, m), 3.62 (3H, s), 5.42 (1H, m), 6.42 (1H, d), 7.22-7.38 (5H, m).

PREPARATION 26

N-[(1*S*)-3-Oxo-1-phenylpropyl]cyclobutanecarboxamide

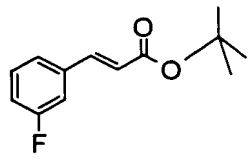


Obtained from the title compound of preparation 25 as a brown oil in 82% yield using a similar procedure to that in preparation 21.

^{1}H-NMR (300MHz, $CDCl_3$) : δ [ppm] 1.81-2.35 (6H, m), 2.90-3.10 (3H, m), 5.53 (1H, m), 5.98 (1H, br d), 7.23-7.39 (5H, m), 9.78 (1H, m).

PREPARATION 27

tert-Butyl (*E*)-3-(3-fluorophenyl)-2-propenoate

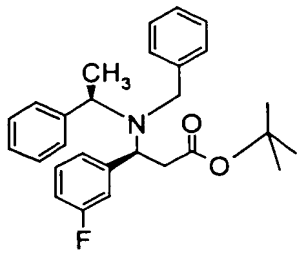


To a solution of 3-fluorobenzaldehyde (10g, 80mmol) in tetrahydrofuran (370ml) was added *tert*-butyl 2-(triphenylphosphoranylidene)acetate (27.6g, 73.3mmol) in 1g portions over 30 minutes. Upon final addition, the mixture was heated to reflux for 10 minutes. The solvent was removed under reduced pressure. The white waxy residue was triturated with pentane (x2). The pentane extracts were combined and evaporated under reduced pressure. The residue was purified by filtration though a plug of silica gel using an eluent of diethyl ether:hexane (1:2) to afford the title compound as a colorless oil, 16.2g.

^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] 1.52 (9H, s), 6.32-6.39 (1H, d), 7.00-7.06 (1H, m), 7.16-7.21 (1H, m), 7.26-7.29 (1H, m), 7.29-7.37 (1H, m), 7.48-7.55 (1H, d).

PREPARATION 28

tert-Butyl (3*S*)-3-{benzyl[(1*R*)-1-phenylethyl]amino}-3-(3-fluorophenyl)propanoate



To a solution of (1*R*)-*N*-benzyl-1-phenyl-1-ethanamine (23.1g, 109.3mmol) in tetrahydrofuran (100ml) at -10°C under an atmosphere of nitrogen gas was added 1.6M n-butyl lithium in hexanes (66ml, 105.7mmol) dropwise. The purple solution was stirred for 15

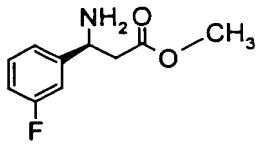
minutes, cooled to -78°C. A solution of the title compound of preparation 27 in tetrahydrofuran (100ml) was added dropwise. After stirring for 30 minutes, the mixture was quenched with 100ml of saturated ammonium chloride solution and stirred to room temperature. The mixture was extracted with diethyl ether (x2). The combined organic layers were dried ($MgSO_4$), filtered and evaporated under reduced pressure. The residue was dissolved in diethyl ether and was washed with 1N citric acid (x2) and then water. The organic layer was dried ($MgSO_4$), filtered and evaporated under reduced pressure. The pale yellow oily residue was purified by column chromatography on silica gel using a gradient elution of diethyl ether:hexane (0:100 to 5:95) to afford the title compound as a colorless oil, 23.0g.

^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] 1.19-1.32 (3H, m), 1.23 (9H, s), 2.42-2.52 (2H, m), 3.68 (2H, s), 3.90-4.00 (1H, m), 4.35-4.42 (1H, m), 6.89-6.97 (1H, m), 7.10-7.35 (11H, m), 7.35-7.42 (2H, m).

LRMS: m/z 434.5 (MH^+)

PREPARATION 29

Methyl (3*S*)-3-amino-3-(3-fluorophenyl)propanoate



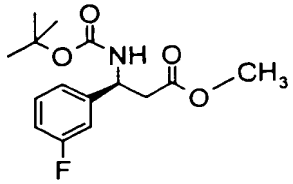
A mixture of the title compound of preparation 28 (23.0g, 53.1mmol), ammonium formate (33.5g, 531mmol) and 20% palladium hydroxide on carbon (12.5g) were heated to reflux for 30 minutes in ethanol (300ml). After cooling to room temperature the mixture was filtered through Arbocel® and the filtrate evaporated under reduced pressure. The residue (16.3g, 68.1mmol) was refluxed for 1.5 hours in a 2.25M solution of anhydrous hydrogen chloride in methanol (150ml). The mixture was evaporated under reduced pressure and the residue was triturated with ethyl acetate to afford the title compound as a white solid, 4.4g.

^{1}H NMR (400 MHz, CD_3OD): δ [ppm] 3.00-3.16 (2H, m), 3.71 (3H, s), 4.74-4.81 (1H, m), 7.13-7.23 (1H, m), 7.24-7.34 (2H, m), 7.44-7.53 (1H, m).

LRMS: m/z 198.2 (MH^+)

PREPARATION 30

Methyl (3*S*)-3-[(*tert*-butoxycarbonyl)amino]-3-(3-fluorophenyl)propanoate

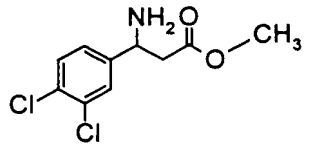


To a suspension of the title compound of preparation 29 (3.8g, 16.3mmol) in tetrahydrofuran (55ml) was added di-tert-butyl dicarbonate (4.26g, 19.5mmol) and 18ml of 2N aqueous sodium hydroxide. The mixture was stirred for 16 hours at room temperature. The mixture was diluted with water and extracted with diethyl ether (x3). The combined organic layers were dried ($MgSO_4$), filtered and evaporated under reduced pressure. The residue was purified by recrystalizing from hexane to afford the title compound as a white solid, 4.1g.

^1H NMR (400 MHz, $CHCl_3$): δ [ppm] 1.40 (9H, s), 2.76-2.89 (2H, m), 3.63 (3H, m), 5.01-5.13 (1H, m), 5.42-5.65 (1H, bs), 6.90-6.97 (1H, m), 6.97-7.02 (1H, m), 7.03-7.10 (1H, m), 7.26-7.32 (1H, m).

PREPARATION 31

Methyl-3-amino-3-(3,4-dichlorophenyl)propanoate



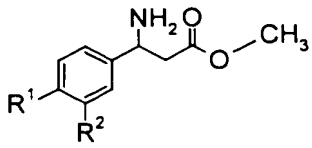
Malonic acid (7.61g, 73.1mmol), 3,4-dichlorobenzaldehyde (12.5g, 71.4mmol) and ammonium acetate (11.2g, 146.3mmol) were heated in ethanol (15ml) at 55°C for 15 hours. The solvent was removed under reduced pressure and the resulting white solid was suspended in methanolic hydrochloric acid (2.25M, 100ml) and heated under reflux for 5 hours. Upon cooling the solvent was removed under reduced pressure and the residue was suspended in water and basified to pH 8 using saturated sodium carbonate solution. The aqueous solution was extracted with dichloromethane (3x) and the combined organic extracts were washed with brine, dried ($MgSO_4$), filtered and evaporated under reduced pressure. The residual brown oil was purified by column chromatography on silica gel using ethyl acetate as eluant to afford the title compound as a cream solid, 5.72g.

^1H-NMR (300MHz, $CDCl_3$) : δ [ppm] 2.61 (2H, m), 3.69 (3H, s), 4.39 (1H, t), 7.20 (1H, d), 7.40 (1H, d), 7.50 (1H, s).

LRMS : m/z 248 (MH^+)

PREPARATIONS 32 TO 34

The compounds of the following tabulated preparations with the general formula;

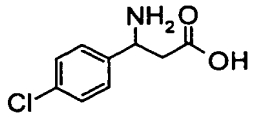


were obtained from the corresponding aldehydes using a similar procedure to that in preparation 31.

PREP. NO.	R^1	R^2	YIELD	CHARACTERIZATION DATA
32	H	F	49%	^{1}H-NMR (300MHz, $CDCl_3$) : δ [ppm] 1.58-1.82 (2H, br m), 2.63 (2H, m), 3.68 (3H, s), 4.42 (1H, m), 6.93 (1H, m), 7.02 (2H, m), 7.28 (1H, m). LRMS : m/z 198 (MH^+)
33	H	Cl	36%	^{1}H-NMR (300MHz, $CDCl_3$) : δ [ppm] 2.54-2.77 (2H, m), 3.69 (3H, s), 4.29-4.55 (1H, m), 7.08-7.35 (3H, m), 7.39 (1H, s). LRMS : m/z 214 (MH^+)
34	OMe	H	27%	^{1}H-NMR (300MHz, $CDCl_3$) : δ [ppm] 1.80 (2H, br s), 2.62 (2H, d), 3.71 (3H, s), 3.83 (3H, s), 4.38 (1H, t), 6.93 (2H, d), 7.23 (2H, d).

PREPARATION 35

3-(4-Chlorophenyl)-β-alanine



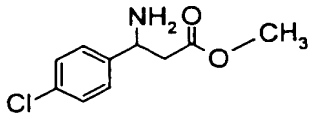
Malonic acid (7.29g, 70.0mmol), 4-chlorobenzaldehyde (9.84g, 70.0mmol) and ammonium acetate (10.9g, 140mmol) were heated in ethanol (15ml) at 55°C for 15 hours. Upon cooling the solvent was removed under reduced pressure and the residue was suspended in water and basified to pH 8 using saturated sodium carbonate solution. The aqueous was extracted with dichloromethane (3x) and the combined organic extracts were washed with brine, dried ($MgSO_4$), filtered and evaporated under reduced pressure to afford the title compound as a cream solid, 3.48g.

^{1}H-NMR (400MHz, CF_3CO_2D) : δ [ppm] 3.28-3.40 (1H, m), 3.52 (1H, m), 5.08 (1H, m), 7.47-7.55 (2H, d), 7.55-7.63 (2H, d), 11.46 (1H, s).

LRMS : m/z 200 (MH^+)

PREPARATION 36

Methyl-3-amino-3-(4-chlorophenyl)propanoate



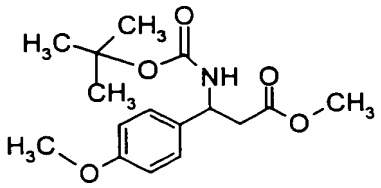
The title compound of preparation 35 (3.48g, 17.47mmol) was suspended in methanolic hydrochloric acid (2.25M, 100ml) and heated under reflux for 5 hours. Upon cooling the solvent was removed under reduced pressure and the residue was suspended in water and basified to pH 8 using saturated sodium carbonate solution. The aqueous was extracted with dichloromethane (3x) and the combined organic extracts were washed with brine, dried ($MgSO_4$), filtered and evaporated under reduced pressure. The residual brown oil was purified by column chromatography on silica gel using ethyl acetate:hexane (80:20) as eluant to afford the title compound as a cream solid, 2.62g.

^{1}H-NMR (400MHz, $CDCl_3$) : δ [ppm] 1.55-1.78 (2H, br s), 2.62 (2H, d), 3.68 (3H, s), 4.37-4.45 (1H, br s), 7.31 (4H, s).

LRMS : m/z 214 (MH^+)

PREPARATION 37

Methyl-3-[(*t*-butoxycarbonyl)amino]-3-(4-methoxyphenyl)propanoate

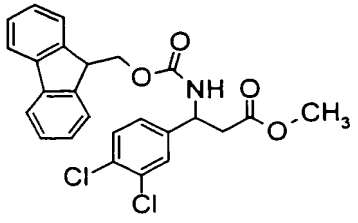


Di-*t*-butyl dicarbonate (2.30g, 10.5mmol) in dichloromethane (5ml) was added dropwise to a solution of triethylamine (1.52ml, 10.5mmol) and the title compound of preparation 34 (2.00g, 9.57mmol) in dichloromethane (20ml) at 0°C. The reaction was allowed to warm to room temperature and stirred for 12 hours. The solvent was removed under reduced pressure and the residue was partitioned between ethyl acetate and water and the layers separated. The organic layer was washed with brine, dried ($MgSO_4$), filtered and the solvent removed under reduced pressure to give a cream solid that was recrystallised from hexane to afford the title compound as a white solid, 2.15g.

^{1}H NMR (300MHz, $CDCl_3$): δ [ppm] 1.42 (9H, s), 2.73-2.94 (2H, m), 3.61 (3H, s), 3.79 (3H, s), 4.97-5.06 (1H, m), 5.23-5.39 (1H, br s), 6.87 (2H, d), 7.19 (2H, d).

PREPARATION 38

Methyl-3-(3,4-dichlorophenyl)-3-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}propanoate



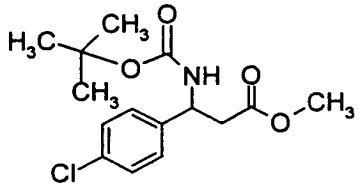
N-(9-Fluorenylmethoxycarbonyloxy)succinimide (7.07g, 20.9mmol) was added to a suspension of the title compound of preparation 31 (5.72g, 23.0mmol) and sodium hydrogencarbonate (1.94g, 23.0mmol) in water (140ml) and acetone (70ml). The reaction stirred at room temperature for 12 hours. The reaction mixture was evaporated under reduced pressure, the residue was partitioned between ethyl acetate and water and the layers separated. The organic phase was washed with brine, dried ($MgSO_4$), filtered and the solvent removed under reduced pressure to afford the title compound as a cream solid, 7.68g.

^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm] 2.70-2.97 (2H, br m), 3.65 (3H, s), 4.11-4.26 (1H, br m), 4.55-4.73 (2H, m), 5.00-5.17 (1H, br m), 5.71-5.92 (1H, br m), 7.01-7.20 (1H, br m), 7.27-7.34 (2H, m), 7.47-7.68 (2H, b), 7.68-7.84 (6H, m).

LRMS: m/z 470 (MH^+)

PREPARATION 39

Methyl-3-[(*t*-butoxycarbonyl)amino]-3-(4-chlorophenyl)propanoate

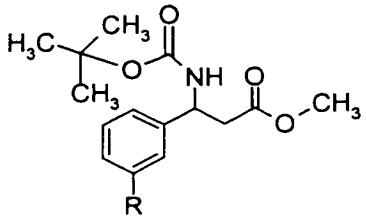


Di-*t*-butyl dicarbonate (11.6g, 53.2mmol) was added to a mixture of 2N sodium hydroxide (25ml) and the title compound of preparation 36 (8.78g, 41.1mmol) in tetrahydrofuran (80ml) at room temperature and stirred for 12 hours. The reaction mixture was evaporated under reduced pressure and the residue was partitioned between ethyl acetate and water and the layers separated. The organic phase was washed with brine, dried ($MgSO_4$), filtered and evaporated under reduced pressure to afford the title compound as a white solid, 12.51g.

^{1}H-NMR (400MHz, $CDCl_3$) : δ [ppm] 1.42 (9H, s), 2.71-2.90 (2H, m), 3.61 (3H, s), 4.98-5.13 (1H, br s), 5.39-5.61 (1H, br m), 7.23 (2H, m), 7.30 (2H, m).

PREPARATIONS 40 TO 41

The compounds of the following tabulated preparations with the general formula:

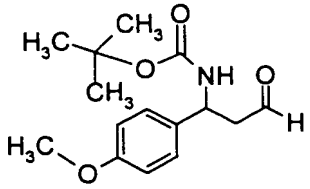


were obtained from the corresponding amines, using a similar procedure to that in preparation 39.

PREP. NO.	R	YIELD	CHARACTERIZATION DATA
40	Cl	100%	^{1}H-NMR (300MHz, $CDCl_3$) : δ [ppm] 1.53 (9H, s), 2.81-2.93 (2H, d), 3.63 (3H, s), 5.08 (1H, br s), 5.56 (1H, br s), 7.20 (4H, m).
41	F	100%	^{1}H-NMR (300MHz, $CDCl_3$) : δ [ppm] 1.42 (9H, s), 2.77-2.89 (2H, d), 3.63 (3H, s), 4.99-5.15 (1H, br s), 6.87-7.18 (3H, m), 7.24-7.35 (1H, m).

PREPARATION 42

t-Butyl-1-(4-methoxyphenyl)-3-oxopropylcarbamate

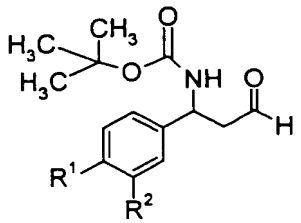


Obtained from the title compound of preparation 37 as a clear oil in 88% yield using a similar procedure to that in preparation 21.

^{1}H NMR (300MHz, $CDCl_3$): δ [ppm] 1.42 (9H, s), 2.82-3.03 (2H, m), 3.81 (3H, s), 4.94-5.03 (1H, br s), 5.06-5.19 (1H, m), 6.87 (2H, d), 7.23 (2H, d), 9.74 (1H, s).

PREPARATIONS 43 TO 45

The compounds of the following tabulated examples with the general formula:



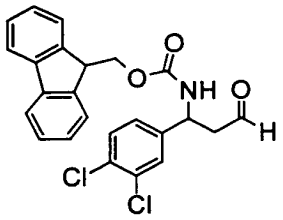
were obtained from the corresponding methyl esters, using a similar procedure to that in preparation 21.

PREP. NO.	R^1	R^2	YIELD	CHARACTERIZATION DATA

43	H	F	83%	^{1}H-NMR (300MHz, $CDCl_3$) : δ [ppm] 1.42 (9H, s), 2.98 (2H, m), 5.03-5.26 (2H, br m), 6.98 (3H, m), 7.30 (1H, m), 9.74 (1H, s).
44	H	Cl	85%	^{1}H-NMR (300MHz, $CDCl_3$) : δ [ppm] 1.43 (9H, s), 2.87-3.03 (2H, br m), 5.09 (2H, br s), 7.20 (4H, m), 9.76 (1H, s).
45	Cl	H	92%	^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm] 1.42 (9H, s), 2.83-3.00 (2H, m), 5.00-5.21 (1H, br m), 7.17-7.28 (4H, m), 9.73 (1H, s).

PREPARATION 46

9*H*-Fluoren-9-ylmethyl-1-(3,4-dichlorophenyl)-3-oxopropylcarbamate



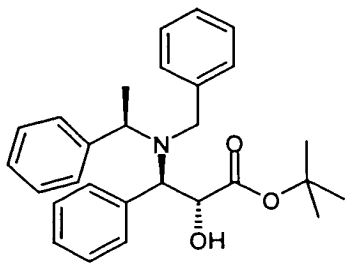
Obtained from the title compound of preparation 38 as a clear oil in 62% yield using a similar procedure to that in preparation 21.

^{1}H-NMR (300MHz, $CDCl_3$) : δ [ppm] 2.85-3.13 (2H, br s), 4.14 (1H, m), 4.53 (2H, m), 5.06-5.24 (1H, br s), 5.28-5.48 (1H, br s), 6.93-7.17 (1H, br s), 7.28 (2H, m), 7.39 (4H, m), 7.58 (2H, m), 7.66 (2H, m), 9.68 (1H, s).

LRMS : m/z 440 (MH^+)

PREPARATION 47

tert-Butyl (2*R*,3*R*)-3-{benzyl[(1*R*)-1-phenylethyl]amino}-2-hydroxy-3-phenylpropanoate



To a solution of (1*R*)-*N*-benzyl-1-phenyl-1-ethanamine (16.6g, 79mmol) in tetrahydrofuran (200ml) at -10 °C was added n-butyl lithium (46ml of a 1.6M solution in hexane, 74mmol) dropwise. The purple solution was stirred for 15 minutes, cooled to -78°C and a solution of *tert*-butylcinnamate (10.0g, 49mmol) in tetrahydrofuran (100ml) added

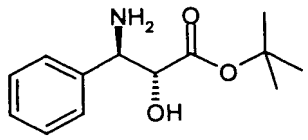
dropwise. After stirring for 2 hours (-)-camphorsulphonyl oxaziridine (18g, 79mmol) was added portionwise and the reaction stirred at -78°C for 1 hour. The reaction was warmed to room temperature and saturated aqueous ammonium chloride solution added and the solvent removed under reduced pressure. The remaining aqueous mixture was extracted with dichloromethane (x2) and the combined organic layers were dried ($MgSO_4$), filtered and evaporated under reduced pressure. The residue was dissolved in diethyl ether, filtered and the filtrate washed with water, dried ($MgSO_4$), filtered and evaporated under reduced pressure. The pale yellow oily residue was purified by column chromatography on silica gel using a gradient elution of diethyl ether:hexane (0:100 to 10:90) to afford the title compound as a colourless oil, 10.0g.

^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm] 1.23 (12H, m), 2.78 (1H, d), 3.81 (1H, d), 4.11 (1H, d), 4.18 (1H, d), 4.20 (1H, d), 4.39 (1H, m), 7.15-7.50 (15H, m)

LRMS: m/z 432.0 (MH^+)

PREPARATION 48

tert-Butyl (2*R*,3*R*)-3-amino-2-hydroxy-3-phenylpropanoate



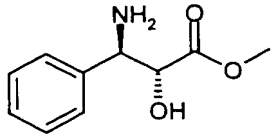
A mixture of the title compound of preparation 47 (650mg, 1.50mmol), ammonium formate (481mg, 7.52mmol) and 20% palladium hydroxide on carbon (500mg) were heated under reflux in ethanol for 30 minutes (10ml). The reaction was cooled and filtered through Arbocel® and the filtrate evaporated under reduced pressure to afford the title compound as a brown oil, 355mg.

^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] 1.23 (9H, s), 4.45 (1H, bs), 4.60 (1H, bs), 7.20-7.35 (5H, m), 8.60 (3H, bs)

LRMS: m/z 238.1 (MH^+)

PREPARATION 49

Methyl (2*R*,3*R*)-3-amino-2-hydroxy-3-phenylpropanoate hydrochloride



The title compound of preparation 48 (1.00g, 4.21mmol) was heated under reflux in methanolic hydrochloric acid (50ml, 2.25M) for 1 hour. The mixture was cooled and the

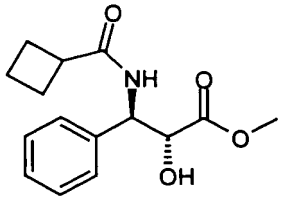
solvent evaporated under reduced pressure to afford the title compound as a brown oil, 950mg.

^{1}H NMR (400 MHz, CD_3OD): δ [ppm] 3.60 (3H, s), 4.65 (1H, bs), 4.78 (1H, bs), 7.38-7.55 (5H, m)

LRMS: m/z 196.9 (MH^+)

PREPARATION 50

Methyl (2*R*,3*R*)-3-[(cyclobutylcarbonyl)amino]-2-hydroxy-3-phenylpropanoate



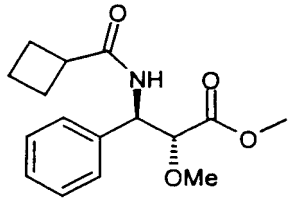
Cyclobutanecarbonyl chloride (0.65ml, 5.19mmol) was added dropwise to a solution of the title compound of preparation 49 (920mg, 4.72mmol) and triethylamine (1.58ml, 11.3mmol) in dichloromethane (20ml) at 0°C. The reaction mixture was stirred for 2 hours at room temperature after which time the mixture was washed with water then brine, dried ($MgSO_4$), filtered and the solvent removed under reduced pressure to afford the title compound as a yellow oil, 1.23g.

^{1}H-NMR (400MHz, $CDCl_3$) : δ [ppm] 2.00 (2H, m), 2.10-2.35 (4H, m), 3.08 (1H, m), 3.25 (1H, m), 3.62 (3H, s), 4.59 (1H, m), 5.42 (1H, dd), 6.30 (1H, d), 7.25-7.35 (5H, m)

LRMS : m/z 279.0 (MH^+)

PREPARATION 51

Methyl (2*R*,3*R*)-3-[(cyclobutylcarbonyl)amino]-2-methoxy-3-phenylpropanoate



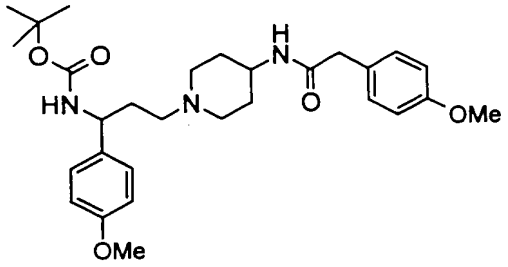
The title compound of preparation 50 (1.00g, 3.61mmol), silver oxide (917mg, 3.97mmol) and iodomethane (1.79ml, 28.8mmol) were heated under reflux in acetonitrile (20ml) for 24 hours. The reaction was cooled and filtered through Arbocel® and the filtrate evaporated under reduced pressure. The residue was purified by flash column chromatography on silica gel using ethyl acetate:hexane (70:30) as eluant to afford the title compound as a white solid, 470mg.

^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] 1.90 (2H, m), 2.10-2.35 (4H, m), 3.08 (1H, m), 3.38 (3H, s), 3.79 (3H, s), 4.07 (1H, d), 5.42 (1H, dd), 6.30 (1H, d), 7.25-7.35 (5H, m)

LRMS: m/z 314.0 (MNa^+)

PREPARATION 52

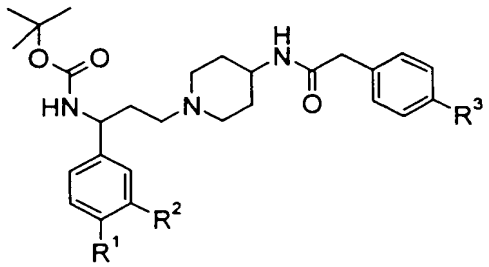
t-Butyl-3-(4-{[2-(4-methoxyphenyl)acetyl]amino}-1-piperidinyl)-1-(4-methoxyphenyl)propylcarbamate



The title compound of preparation 6 (444mg, 1.79mmol) was dissolved in dichloromethane/acetic acid (20ml, 10% solution) and the title compound of preparation 42 (500mg, 1.79mmol) was added followed by sodium triacetoxyborohydride (570mg, 2.69mmol). The reaction mixture was stirred for 18 hours after which time the solution was basified using saturated aqueous sodium carbonate and the product was extracted using dichloromethane (x3). The combined organic extracts were dried ($MgSO_4$) and the solvent evaporated under reduced pressure to give a brown oil. This was purified by column chromatography on silica gel using dichloromethane:methanol:0.88 ammonia (98:2:0.25) as eluant to afford the title compound as a clear oil, 800mg.

PREPARATIONS 53-55

The compounds of the following tabulated examples with the general formula:

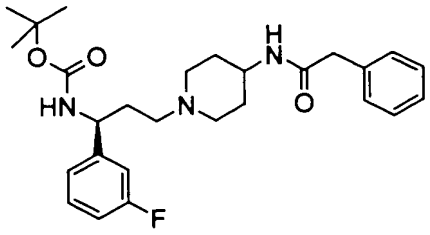


were obtained from the corresponding aldehydes, using a similar procedure to that in preparation 52.

PREP. NO.	R1	R2	R3	YIELD	CHARACTERIZATION DATA
53	H	Cl	H		^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] 1.18-1.42 (11H, m), 1.66 (1H, bs), 1.92-2.16 (5H, m), 2.20-2.36 (2H, m), 2.63 (1H, bs), 2.79 (1H, bs), 3.58 (2H, s), 3.80 (1H, m), 4.73 (1H, bs), 5.18 (1H, bs), 6.80 (1H, bs), 7.08 (1H, d), 7.11-7.28 (8H, m). LRMS: m/z 486 (MH^+)
54	H	F	H		^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] 1.20-1.45 (11H, bs), 1.61-1.77 (1H, bs), 1.82-2.14 (5H, m), 2.18-2.37 (2H, m), 2.55-2.70 (1H, bs), 2.71-2.94 (1H, bs), 3.55 (2H, s), 3.71-3.85 (1H, m), 4.66-4.83 (1H, bs), 5.06-5.26 (1H, bs), 6.72-6.85 (1H, m), 6.85-7.05 (3H, m), 7.23-7.40 (6H, m). LRMS: m/z 470 (MH^+)
55	Cl	H	H		^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] 1.20-1.45 (10H, bs), 1.60-1.79 (1H, bs), 1.82-2.15 (6H, m), 2.19-2.36 (2H, m), 2.55-2.71 (1H, bs), 2.71-2.94 (1H, bs), 3.56 (2H, s), 3.71-3.87 (1H, bm), 4.60-4.80 (1H, bs), 5.09-5.25 (1H, bs), 6.67-6.85 (1H, bs), 7.18 (1H, d), 7.20-7.40 (8H, m). LRMS: m/z 486 (MH^+)

PREPARATION 56

tert-Butyl (1*S*)-1-(3-fluorophenyl)-3-{4-[(2-phenylacetyl)amino]-1-piperidinyl}propylcarbamate



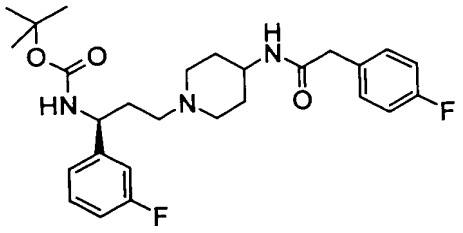
To a solution of the title compound of preparation 30 (1.64g, 5.52mmol) in dichloromethane (30ml) at -78°C under an atmosphere of nitrogen was added dropwise a 1M solution of diisobutylaluminium hydride in dichloromethane (11.6ml, 11.6mmol). After 30 minutes the mixture was quenched with methanol at -78°C (4ml). The mixture was poured in 30ml of 2M aqueous hydrochloric acid and extracted with dichloromethane (x2). The combined organic layers were dried ($MgSO_4$), filtered and evaporated under reduced pressure. The residue (735mg, 2.75mmol), the title compound of preparation 2 (600mg, 2.75mmol) and sodium triacetoxyborohydride (875mg, 4.12mmol) were stirred together for 20 minutes in glacial a 30ml mixture of acetic acid:dichloromethane (1:10). The solvents were removed under reduced pressure and the residue basified with saturated sodium carbonate solution before extracting with dichloromethane (x2). The organic layers were combined and dried ($MgSO_4$), filtered and evaporated under reduced pressure to afford the title compound as a white foam, 1.20g.

^{1}H NMR (400 MHz, $CHCl_3$): δ [ppm] 1.24-1.40 (11H, m), 1.81-2.11 (6H, m), 2.18-2.32 (2H, m), 2.58-2.69 (1H, m), 2.69-2.85 (1H, m), 3.55 (2H, s), 3.71-3.85 (1H, m), 4.68-4.80 (1H, m), 5.13-5.24 (1H, m), 6.73-6.84 (1H, m), 6.84-6.97 (2H, m), 6.97-7.03 (1H, m), 7.20-7.39 (6H, m).

LRMS: m/z 470.1 (MH+)

PREPARATION 57

tert-Butyl(1*S*)-1-(3-fluorophenyl)-3-(4-{[2-(4-fluorophenyl)acetyl]amino}-1-piperidinyl)propylcarbamate

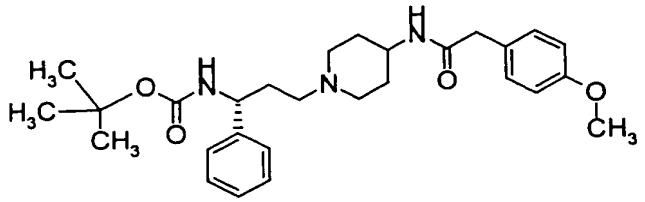


To a solution of the title compound of preparation 30 (2.52g, 8.48mmol) in dichloromethane (50ml) at -78°C under an atmosphere of nitrogen was added dropwise a 1M solution of diisobutylaluminium hydride in dichloromethane (17.8g, 17.8mmol). After 30 minutes the mixture was quenched with methanol at -78°C (7ml). The mixture was poured in 40ml of 2M aqueous hydrochloric acid and extracted with dichloromethane (x2). The combined organic layers were dried ($MgSO_4$), filtered and evaporated under reduced pressure. The residue (1.13g, 4.23mmol), the title compound of preparation 4 (1g, 4.23mmol) and sodium triacetoxyborohydride (1.35g, 6.37mmol) were stirred together for 30 minutes in a 20ml mixture of glacial acetic acid:dichloromethane (1:10). The mixture was evaporated under reduced pressure and basified with saturated sodium carbonate solution before extracting with ethyl acetate. The organic layer was washed with water, dried ($MgSO_4$), filtered and evaporated under reduced pressure to afford the title compound as a foam, 1.73g.

^1H NMR (300 MHz, $CDCl_3$): δ [ppm] 1.29-1.48 (9H, m), 1.60-2.15 (8H, m), 2.20-2.40 (2H, m), 2.60-2.79 (1H, m), 2.79-2.90 (1H, m), 3.50 (2H, s), 3.65-3.87 (2H, m), 4.68-4.82 (1H, m), 5.13-5.29 (1H, m), 6.65-6.80 (1H, m), 6.85-7.10 (4H, m), 7.16-7.35 (3H, m).

PREPARATION 58

t-Butyl (1*R*)-3-(4-{[2-(4-methoxyphenyl)acetyl]amino}-1-piperidinyl)-1-phenylpropylcarbamate



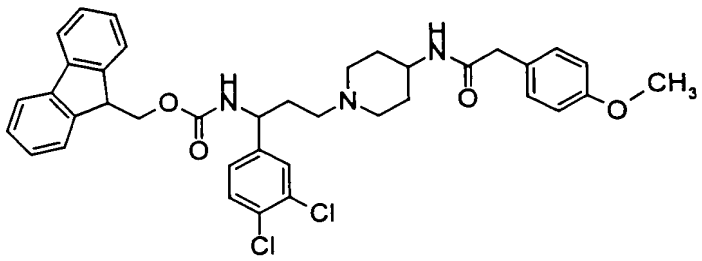
Diisobutylaluminium hydride (24.0ml of a 1.0M solution in dichloromethane, 24.0mmol) was added dropwise to a solution of the title compound of preparation 23 (3.2g, 11.1mmol) in dichloromethane (100ml) at -78°C. The reaction mixture was stirred at this temperature for a further 1 hour. Methanol (5ml) precooled to -78°C was then added and the mixture was warmed to room temperature and washed with 2N hydrochloric acid, water, brine, dried ($MgSO_4$), filtered and the solvent removed under reduced pressure to afford a yellow oil. The oil was dissolved in dichloromethane/acetic acid (50ml, 10% solution) and the title compound of preparation 9 (3.0g, 12.2mmol) was added followed by sodium

triacetoxyborohydride (3.5g, 16.7mmol). The reaction mixture was stirred for 30 minutes after which time the solution was basified using saturated aqueous sodium carbonate and the product was extracted using dichloromethane (x3). The combined organic extracts were dried ($MgSO_4$) and the solvent evaporated under reduced pressure to give a brown oil. This was purified by column chromatography on silica gel using dichloromethane:methanol:0.88 ammonia (95:5:0.5) as eluant to afford the title compound as a clear oil, 4.2g.

LRMS: m/z 482 (MH^+)

PREPARATION 59

9*H*-Fluoren-9-ylmethyl 1-(3,4-dichlorophenyl)-3-(4-{[2-(4-methoxyphenyl)acetyl]amino}-1-piperidinyl)propylcarbamate



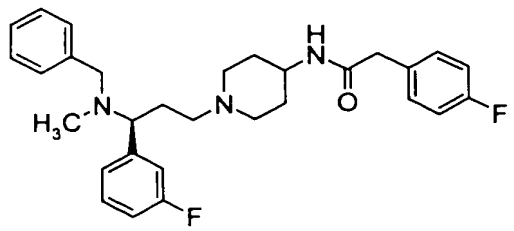
Obtained from the title compounds of preparation 46 and preparation 9 as a clear oil in 9% yield using a similar procedure to that in preparation 52.

^1H NMR (300 MHz, $CDCl_3$): δ [ppm] 1.15-1.42 (2H, m), 1.46-1.76 (2H, m), 1.80-2.16 (5H, m), 2.26 (2H, m), 2.60-2.90 (2H, m), 3.48 (2H, s), 2.60-2.88 (4H, m), 4.16 (1H, m), 4.32-4.56 (2H, m), 4.76 (1H, m), 5.19 (1H, m), 6.69-6.92 (3H, m), 6.94-7.21 (5H, m), 7.26-7.45 (4H, m), 7.57 (2H, m), 7.64-7.82 (1H, m).

LRMS: m/z 672 (MH^+)

PREPARATION 60

N-{1-[(3*S*)-3-[Benzyl(methyl)amino]-3-(3-fluorophenyl)propyl]-4-piperidinyl}-2-(4-fluorophenyl)acetamide



The title compound of preparation 57 (1.73g, 3.55mmol) was stirred for 30 minutes in a mixture of trifluoroacetic acid:dichloromethane (34ml, 1:1). The mixture was evaporated under reduced pressure and basified with saturated sodium carbonate solution before

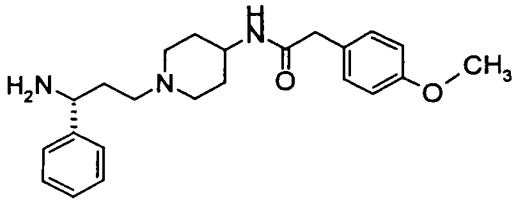
extracting with dichloromethane (x3). The organic layers were combined and dried ($MgSO_4$), filtered and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel using an eluent of dichloromethane:methaniol:0.88 ammonia (90:10:1). The residue (250mg, 0.65mmol), benzaldehyde (70mg, 0.65mmol) and sodium triacetoxyborohydride (175mg, 0.78mmol) were stirred together for 15 minutes in a mixture of glacial acetic acid:dichloromethane (10 ml, 1:10). The mixture was evaporated under reduced pressure and basified with saturated sodium carbonate solution before extracting with dichloromethane (x3). The organic layers were combined and dried ($MgSO_4$), filtered and evaporated under reduced pressure. The residue was heated for 1 hour at 100 °C. in a 6ml mixture of formic acid:formaldehyde (1:2). The mixture was evaporated under reduced pressure and basified with saturated sodium carbonate solution before extracting with dichloromethane (x3). the organic layers were combined and dried ($MgSO_4$), filtered and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol:0.88 ammonia (98:2:0.25 to 95:5:0.5) to afford the title compound an a gum, 260mg.

^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] 1.23-1.58 (2H, m), 1.81-1.90 (3H, m), 1.94-2.21 (4H, m), 2.06 (3H, s), 2.26-2.35 (1H, m), 2.61-2.74 (2H, m), 3.20-3.26 (1H, m), 3.48 (2H, s), 3.49-3.60 (2H, m), 3.68-3.80 (1H, m), 5.11-5.20 (1H, m), 6.90-7.06 (5H, m), 7.16-7.32 (8H, m).

LRMS: m/z 492.2 (MH+)

PREPARATION 61

N-{1-[(3*R*)-3-amino-3-phenylpropyl]-4-piperidinyl}-2-(4-methoxyphenyl)acetamide



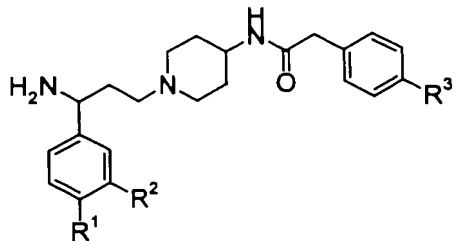
Trifluoroacetic acid (2ml) was added dropwise to a solution of the title compound of preparation 58 (670mg, 1.39mmol) in dichloromethane (10ml). The reaction was stirred at room temperature for 0.5 hours, then heated to reflux for 15 minutes. After cooling to room temperature more trifluoroacetic acid (2ml) was added and the reaction was refluxed for further 10 minutes. After cooling to room temperature the reaction mixture was concentrated under reduced pressure. The residue was dissolved in dichloromethane, then washed with aqueous sodium carbonate solution. The organic layer was dried ($MgSO_4$), filtered and evaporated under reduced pressure. The residue was purified by column chromatography on

silica gel, using an elution gradient of dichloromethane:methanol:0.88 ammonia (95:5:0.5 to 90:10:1) to afford the title compound as a white solid, 200mg.

^{1}H NMR (400MHz, $CDCl_3$): δ [ppm] 1.23-1.35 (2H, m), 1.74-1.87 (4H, m), 1.94-2.06 (2H, m), 2.20-2.29 (1H, m), 2.29-2.37 (1H, m), 2.64-2.77 (2H, m), 3.48 (2H, s), 3.68-3.84 (1H, m), 3.81 (3H, s), 3.94 (1H, m), 5.18 (1H, d), 6.87 (2H, d), 7.13 (2H, d), 7.19-7.35 (5H, m).

PREPARATIONS 62 TO 65

The compounds of the following tabulated examples with the general formula:

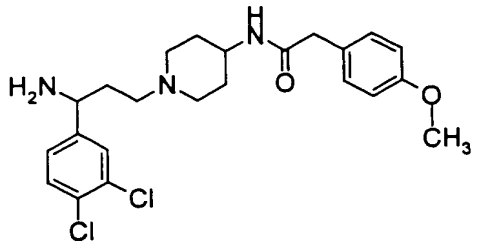


were obtained from the corresponding *t*-butyl carbamates, using a similar procedure to that in preparation 61.

PREP. NO.	R1	R2	R3	YIELD	CHARACTERIZATION DATA
62	OMe	H	OMe	64%	^{1}H NMR (400MHz, $CDCl_3$): δ [ppm] 1.23-1.35 (2H, m), 1.71-1.87 (4H, m), 1.94-2.06 (2H, m), 2.16-2.26 (1H, m), 2.26-2.35 (1H, m), 2.65-2.77 (2H, m), 3.47 (1H, m), 3.69-3.80 (1H, m), 3.77 (3H, s), 3.81 (3H, s), 3.89 (1H, t), 5.20 (1H, d), 6.81-6.90 (4H, m), 7.13 (2H, d), 7.19 (2H, d).
63	H	Cl	H	73%	^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] 1.42 (2H, m), 1.91 (4H, m), 2.06 (2H, m), 2.40 (2H, m), 2.60 (2H, br s), 2.78 (2H, br m), 3.58 (2H, s), 3.79 (1H, m), 3.98 (1H, t), 5.38 (1H, br d), 7.10-7.28 (9H, m). LRMS: m/z 386 (MH^+)
64	H	F	H	79%	^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] 1.20-1.35 (3H, m), 1.68-1.89 (5H, m), 2.02 (2H, q), 2.30 (2H, m), 2.70 (2H, t), 3.35 (2H, s), 3.76 (1H, m), 3.95 (1H, t), 5.17 (1H, d), 6.85-6.94 (1H, m), 6.97-7.09 (2H, m), 7.20-7.39 (6H, m). LRMS: m/z 369 (MH^+)
65	Cl	H	H	69%	^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] 1.29 (2H, m), 1.65-1.90 (4H, m), 2.00 (2H, m), 2.19-2.47 (2H, m), 2.68 (2H, br t), 3.53 (2H, s), 3.76 (1H, br m), 3.94 (1H, t), 5.16 (1H, d), 7.11-7.40 (9H, m). LRMS: m/z 386 (MH^+)

PREPARATION 66

N-{1-[3-Amino-3-(3,4-dichlorophenyl)propyl]-4-piperidinyl}-2-(4-methoxyphenyl)acetamide



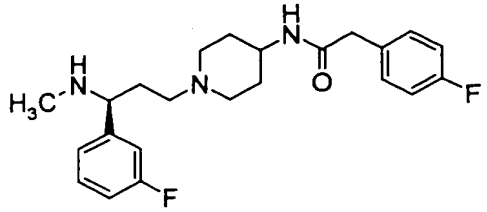
The title compound of preparation 59 (203mg, 0.302mmol) was dissolved in piperidine/dimethylformamide (0.65ml, 15% solution) and dichloromethane (3ml) and reaction stirred at room temperature for 18 hours. The solvent was the evaporated under reduced pressure and the residue partitioned between water and dichloromethane. The aqueous layer was further extracted with dichloromethane (x2), the combined organic layers were dried ($MgSO_4$), filtered and the solvent removed under reduced pressure. The crude product was purified by column chromatography on silica gel using dichloromethane:methanol:0.88 ammonia, (98:2:0.25) as eluant to afford the title compound as a clear oil, 80mg.

^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm] 1.20-1.41 (2H, m), 1.60-1.94 (4H, m), 2.04 (2H, m), 2.31 (2H, m), 2.72 (2H, t), 3.49 (2H, s), 3.68-3.89 (4H, m), 3.96 (1H, t), 5.19 (1H, d), 6.90 (2H, d), 7.14 (3H, d), 7.36 (1H, d), 7.44 (1H, s).

LRMS: m/z 450 (MH^+)

PREPARATION 67

2-(4-Fluorophenyl)-*N*-{1-[(3*S*)-3-(3-fluorophenyl)-3-(methylamino)propyl]-4-piperidinyl}acetamide



The title compound of preparation 60 (260mg, 0.53mmol), ammonium formate (260mg, 4.12mmol) and 20% palladium hydroxide on carbon (50mg) were refluxed together in 10ml of ethanol until gas evolution ceased. After cooling to room temperature, the fixture was filtered though a pad of Arbocel® to removed the catalyst and the filtrate evaporated under reduced pressure to afford the title compound as a gum, 200mg.

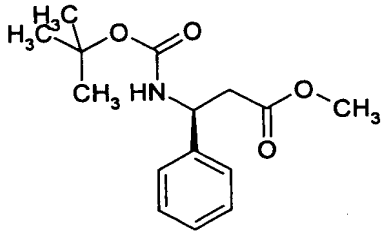
^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] 1.29-1.45 (2H, m), 1.68-1.81 (1H, m), 1.81-1.92 (2H, m), 1.92-2.03 (2H, m), 2.03-2.19 (2H, m), 2.23-2.31 (1H, m), 2.27 (3H, s), 2.31-2.40 (1H,

m), 2.66-2.76 (1H, m), 2.82-2.90 (1H, m), 3.48 (2H, s), 3.56-3.63 (1H, m), 3.68-3.84 (1H, m), 5.29-5.40 (1H, m), 6.89-7.00 (1H, m), 7.00-7.06 (3H, m), 7.06-7.11 (1H, m), 7.18-7.32 (3H, m).

LRMS: m/z 402.1 (MH+)

PREPARATION 68

Methyl (3S)-3-[(*tert*-butoxycarbonyl)amino]-3-phenylpropanoate



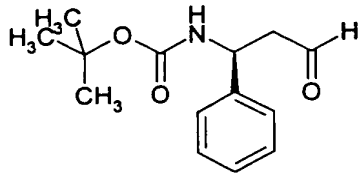
The title compound from preparation 24 (5.38g, 30mmol) and di-*tert*-butyl dicarbonate (8.72g, 40mmol) in tetrahydrofuran (50ml) and 2N sodium hydroxide solution (25ml) were stirred at room temperature for 2 hours. The reaction mixture was diluted with ethyl acetate, the layers separated and the aqueous phase extracted with ethyl acetate (2x). The combined organic solutions were washed with water, brine, dried ($MgSO_4$), filtered and evaporated under reduced pressure to afford the title compound as a white solid, 8.39g.

^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] 1.41 (9H, s), 2.84 (2H, m), 3.61 (3H, s), 5.10 (1H, bs), 5.41 (1H, bs), 7.22-7.36 (5H, m).

LRMS: m/z 279.7 (MH^+)

PREPARATION 69

tert-Butyl (1S)-3-oxo-1-phenylpropylcarbamate



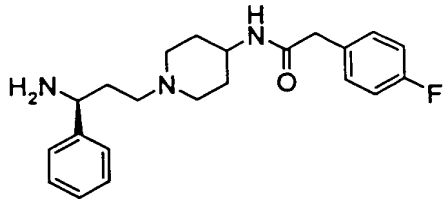
Diisobutylaluminium hydride (1M in dichloromethane, 60ml, 60mmol) was cooled to -78°C and added dropwise to a solution of the title compound from preparation 68 (8.39g, 30mmol) in dichloromethane (150ml) at -78°C. The reaction was stirred for 90 minutes, then methanol (pre-cooled to -78°C, 40ml) was added. The mixture was allowed to warm to room temperature and poured into 2M hydrochloric acid (200ml). The layers were separated and the aqueous phase extracted with dichloromethane (2x). The combined organic layers were dried ($MgSO_4$), filtered and evaporated under reduced pressure to afford the title compound as a white solid, 6.72g.

^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] 1.42 (9H, s), 2.86-3.00 (2H, m), 5.06 (1H, bs), 5.20 (1H, bs), 7.22-7.38 (5H, m), 9.75 (1H, s).

LRMS: m/z 250.1 (MH^+).

PREPARATION 70

N-{1-[(3*S*)-3-Amino-3-phenylpropyl]-4-piperidinyl}-2-(4-fluorophenyl)acetamide

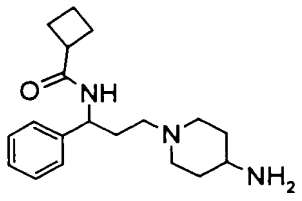


The title compound of preparation 69 (1.4g, 5.62mmol), the title compound of preparation 4 (1.45g, 5.62mmol) and sodium triacetoxyborohydride (1.9g, 8.43mmol) were stirred together for 18 hours in a 50ml mixture of glacial acetic acid: dichloromethane(1:10). The mixture was evaporated under reduced pressure and basified with saturated sodium carbonate solution before extracting with dichloromethane (x3). The organics layers were combined and dried ($MgSO_4$), filtered and evaporated under reduced pressure. The residue was stirred for 2 hours in a 30ml mixture of trifluoroacetic acid:dichloromethane (1:2). The mixture was evaported under reduced pressure and basified with saturated sodium carbonate before extracting with dichloromethane (x5). The organic layers were combined and dried ($MgSO_4$), filtered and evaporated under reduced pressure. The residue was purified by recrystalization from methyl t-butyl ether to afford the title compound as a crystalline solid, 1.55g.

^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] 1.23-1.40 (2H, m), 1.74-2.10 (8H, m), 2.23-2.40 (2H, m), 2.66-2.80 (2H, m), 3.50 (2H ,s), 3.68-3.80 (1H, m), 3.90-3.97 (1H, m) 5.20-5.27 (1H, m), 6.97-7.06 (2H, m), 7.16-7.37 (7H, m).

PREPARATION 71

N-[3-(4-Amino-1-piperidinyl)-1-phenylpropyl]cyclobutanecarboxamide



Sodium triacetoxyborohydride (3.02g, 14.2mmol) and acetic acid (0.54ml, 9.52mmol) was added to a solution of 2,2,2-trifluoro-*N*-(4-piperidinyl)acetamide (1.86g, 9.52mmol) [J. Med. Chem., (1991), 34(2), 656-663] and the title compound of preparation 21 (2.20g,

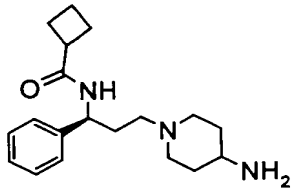
9.52mmol) in 1,1,1-trichloroethane (30ml). The reaction mixture was stirred at room temperature for 18 hours, after which time 2N sodium hydroxide (30ml) and ethanol (30ml) were added to the mixture and stirring continued for 1 hour. The solvent was then removed under reduced pressure and the residue partitioned between water and dichloromethane, the aqueous layer was separated and extracted with dichloromethane (x2). The combined organic layers were dried ($MgSO_4$), filtered and the solvent removed under reduced pressure. The crude product was purified by column chromatography on silica gel using dichloromethane:methanol:0.88 ammonia (90:10:1) as the eluant to afford the title compound as a yellow oil, 1.50g.

^{1}H-NMR (400MHz, $CDCl_3$) : δ [ppm] 1.30-2.40 (18H, m), 2.72 (1H, m), 2.80 (1H, d), 2.92-3.08 (2H, m), 5.13 (1H, m), 7.20-7.33 (5H, m), 7.80 (1H, d).

LRMS : m/z 316 (MH^+)

PREPARATION 72

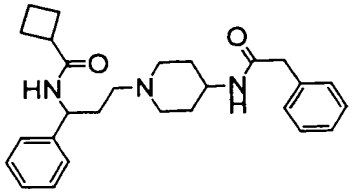
N-(1*S*)-[3-(4-Amino-1-piperidinyl)-1-phenylpropyl]cyclobutanecarboxamide



Obtained from the title compound of preparation 26 and 2,2,2-trifluoro-*N*-(4-piperidinyl)acetamide using a similar procedure to that described for the racemate in preparation 71.

EXAMPLE 1

N-(1-Phenyl-3-{4-[(2-phenylacetyl)amino]-1-piperidinyl}propyl)cyclobutanecarboxamide



Benzoyl chloride (70μl, 0.53mmol) was added to a solution of the title compound of preparation 71 (140mg, 0.44mmol) and triethylamine (74μl, 0.53mmol) in dichloromethane (10ml). The reaction mixture was stirred for 1 hour and purified directly by column

chromatography on silica gel using dichloromethane:methanol:0.88ammonia (95:5:0.5) as eluant to afford the title compound as a white foam, 49mg.

Found C, 73.71; H, 8.20; N, 9.54%

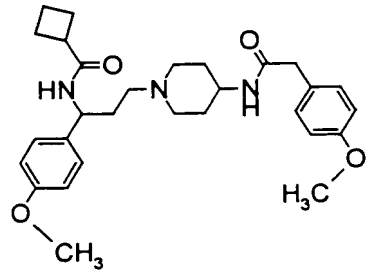
$C_{27}H_{35}N_3O_2$; requires C 74.79; H, 8.14; N, 9.69%

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] 1.21-1.46 (2H, m), 1.73-2.40 (15H, m), 2.69 (1H, d), 2.78 (1H, d), 2.98 (1H, m), 3.56 (2H, s), 3.80 (1H, m), 5.08 (1H, m), 5.25 (1H, d), 7.18-7.42 (10H, m)

LRMS: m/z 434 (MH^+)

EXAMPLE 2

N-[3-(4-{[2-(4-Methoxyphenyl)acetyl]amino}-1-piperidinyl)-1-(4-methoxyphenylpropyl]cyclobutanecarboxamide



Cyclobutanecarbonyl chloride (42µl, 0.37mmol) was added to a solution of the title compound of preparation 62 (150mg, 0.36mmol) and triethylamine (54 µl, 0.39mmol) in dichloromethane (6ml) under ice cooling. The reaction mixture was allowed to warm to room temperature, stirred for 1 hour, then diluted with dichloromethane and water. The layers were separated and the aqueous layer extracted several times with dichloromethane. The combined organic layers were dried ($MgSO_4$), filtered and evaporated under reduced pressure. Purification by column chromatography on silica gel using dichloromethane:methanol: 0.88 ammonia (95:5:0.5) afforded the title compound as a white solid which was recrystallised from ethyl acetate/isopropyl alcohol, 49mg.

Found: C, 70.47; H, 8.00; N, 8.55%

$C_{29}H_{39}N_3O_4$; requires C, 70.56; H, 7.96; N, 8.31%

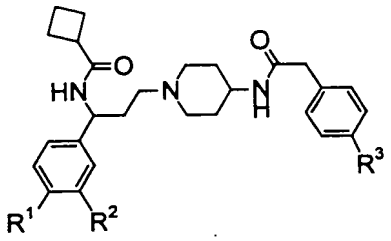
^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] 1.24-1.40 (2H, m); 1.74-2.00 (6H, m), 2.00-2.16 (4H, m), 2.16-2.35 (4H, m), 2.63-2.71 (1H, m), 2.71-2.80 (1H, m), 2.94 (1H, m), 3.50 (2H, s), 3.77 (3H, s), 3.80 (3H, s), 3.73-3.84 (1H, m), 5.02 (1H, m), 5.20 (1H, d), 6.84 (2H, d), 6.90 (2H, d), 7.03 (1H, d), 7.10-7.19 (4H, m)

LRMS: m/z 494 (MH^+)

Melting point [°C]: 165-166 (ethyl acetate/isopropyl alcohol)

EXAMPLE 3-6

The compounds of the following tabulated examples with the general formula:



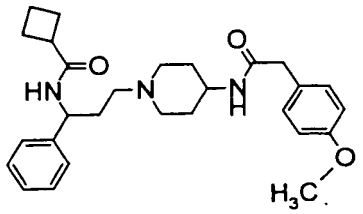
were prepared using a similar method to Example 2 from cyclobutanecarbonyl chloride and the corresponding amines.

EX. NO.	R^1	R^2	R^3	YIELD (%)	CHARACTERIZATION DATA
3	Cl	Cl	OCH_3	85	Found: C, 60.71; H, 6.52; N, 7.45% $C_{28}H_{35}Cl_2N_3O_3;1.1H_2O$ requires C, 60.71; H, 6.51; N 7.45% ^1H NMR (300 MHz, $CDCl_3$): δ [ppm] 1.20 - 1.44 (1H, m); 1.64 - 2.40 (11H, m); 2.60 - 2.86 (2H, m); 2.98 (1H, m); 3.51 (2H, s); 3.70 - 3.80 (1H, m); 3.83 (3H, s); 4.99 (1H, m); 5.23 (1H, d); 6.90 (2H, d); 7.05 (1H, m); 7.18 (2H, d); 7.26 (1H, d); 7.44 (1H, m). LRMS: m/z 532 (MH^+) Melting point [°C]: 80-81
4	H	Cl	H	62	Found: C, 68.57; H, 7.37; N, 8.80% $C_{27}H_{34}N_3O_2Cl;0.3H_2O$; requires C, 68.50; H, 7.37; N 8.88% ^1H NMR (300 MHz, CD_3OD): δ [ppm] 1.43-1.58 (2H, m), 1.79-2.40 (15H, m), 2.81-2.90 (2H, br m), 3.13 (1H, m), 3.46 (2H, s), 3.60-3.68 (1H, broad m), 4.87 (1H, t), 7.18-7.35 (9H, m)

5	H	F	H	48	Found: C, 68.94; H, 7.38; N, 9.03% $C_{27}H_{34}FN_3O_2;H_2O$ requires C, 69.06; H, 7.73; N 8.95% ^1H NMR (400 MHz, $CDCl_3$): δ [ppm] 1.21-1.45 (2H, m), 1.68-1.80 (1H, m), 1.80-2.37 (14H, m), 2.55-2.71 (1H, m), 2.71-2.87 (1H, m), 2.97 (1H, m), 3.57 (2H, s), 3.80 (1H, m), 5.03-5.12 (1H, m), 5.18-5.27 (1H, m), 6.83-6.94 (2H, m), 6.99 (1H, d), 7.19-7.27 (2H, m), 7.27-7.42 (3H, m), 7.58 (1H, d) LRMS: m/z 452.1 (MH^+) Melting point [°C]: 160
6	Cl	H	H	61	Found: C, 68.05; H, 7.41; N, 8.81% $C_{27}H_{33}FN_4O;0.5H_2O$ requires C, 67.98; H, 7.40; N 8.81% ^1H NMR (400 MHz, $CDCl_3$): δ [ppm] 1.32 (2H, m), 1.66-1.80 (1H, m), 1.80-2.35 (13H, m), 2.57-2.70 (1H, br d), 2.70-2.81 (1H, br d), 2.97 (1H, d), 3.58 (2H, s), 3.81 (1H, m), 5.04 (1H, m), 5.20 (1H, d), 7.15 (2H, d), 7.24-7.40 (6H, m), 7.43-7.56 (1H, d) LRMS: m/z 468.0 (MH^+) Melting point [°C]: 70-71

EXAMPLE 7

N-[3-(4-{[2-(4-Methoxyphenyl)acetyl]amino}-1-piperidinyl)-1-phenylpropyl]cyclobutanecarboxamide



1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (109mg, 0.57mmol) was added to a solution of the title compound of preparation 71 (150mg, 0.47mmol), 2-(4-methoxyphenyl)acetic acid (7.9mg, 0.47mmol) and 1-hydroxybenzotriazole hydrate (80mg,

0.52mmol) in dichloromethane (10ml). The reaction mixture was stirred for 56 hours after which time the mixture was washed with water and the organic layer separated and dried ($MgSO_4$). The crude product was purified by column chromatography on silica gel using dichloromethane:methanol:0.88 ammonia (95:5:0.5) as eluant to afford the title compound as a white foam, 60mg.

Found: C, 71.72; H, 8.07; N, 9.02%

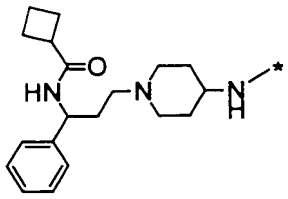
$C_{28}H_{37}N_3O_3x0.3H_2O$; requires C, 71.70; H, 8.07; N, 8.96%

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] 1.20-1.42 (2H, m), 1.72-2.36 (15H, m), 2.67 (1H, d), 2.78 (1H, d), 2.98 (1H, m), 3.49 (2H, s), 3.72-3.85 (4H, m), 5.08 (1H, m), 5.25 (1H, d), 6.89 (2H, d), 7.12-7.34 (7H, m)

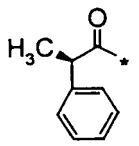
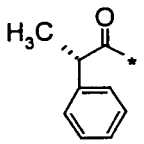
LRMS: m/z 464 (MH^+)

EXAMPLE 8-9

The compounds of the following tabulated examples with the general formula:

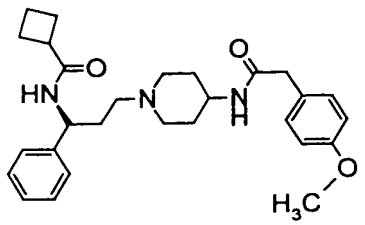


were prepared using a similar method to Example 7 from the title compound of preparation 71 and the corresponding acids.

EX. NO.	R	YIELD (%)	CHARACTERIZATION DATA
8		37	Found: C, 73.98; H, 8.42; N, 9.13% $C_{28}H_{37}N_3O_2$;0.4H_2O; requires C, 73.94; H, 8.38; N 9.24% ^{1}H NMR (300 MHz, $CDCl_3$): mixture of diastereomers δ [ppm] 1.17-1.42 (2H, m), 1.48 and 1.50 (3H, 2xs), 1.72-2.38 (15H, m), 2.57-2.82 (2H, br m), 2.96 (1H, m), 3.54 (1H, m), 3.68-3.82 (1H, br m), 5.01-5.18 (2H, m), 7.13-7.40 (10H, m) LRMS: m/z 448.5 (MH^+)
9		53	Found: C, 69.90; H, 7.77; N, 8.65% $C_{28}H_{37}N_3O_2$;0.5CH_2Cl_2; requires C, 69.85; H, 7.82; N 8.57% ^{1}H NMR (300 MHz, $CDCl_3$): mixture of diastereomers δ [ppm] 1.18-1.41 (2H, m), 1.45 and 1.50 (3H, 2xs), 1.72-2.40 (15H, m), 2.58-2.81 (2H, br m), 2.97 (1H, m), 3.50 (1H, m), 3.67-3.83 (1H, br m), 5.00-5.20 (2H, m), 7.12-7.39 (10H, m) LRMS: m/z 448.9 (MH^+)

EXAMPLE 10

N-[(1*S*)-3-(4-{[2-(4-Methoxyphenyl)acetyl]amino}-1-piperidinyl)-1-phenylpropyl]cyclobutanecarboxamide



The title compound was prepared using a similar method to preparation 71 from the title compounds of preparations 26 and 6. The crude product was purified by column chromatography on silica gel using dichloromethane:methanol:0.88 ammonia (98:2:0.25) as the eluant, and the product was then recrystallised from ethyl acetate to afford the title compound as a solid, 90mg.

Found: C, 72.39; H, 8.04; N, 9.01%

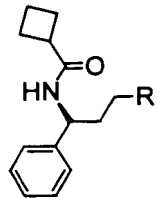
$C_{28}H_{37}N_3O_3$; requires C, 72.54; H, 8.04; N, 9.06%

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] 1.21-1.43 (2H, m), 1.75-2.37 (15H, m), 2.65 (1H, d), 2.78 (1H, d), 2.97 (1H, m), 3.49 (2H, s), 3.70-3.85 (4H, m), 5.09 (1H, m), 5.21 (1H, d), 6.88 (2H, d), 7.13-7.35 (7H, m)

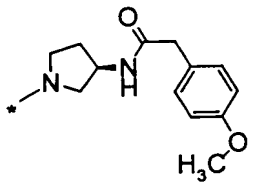
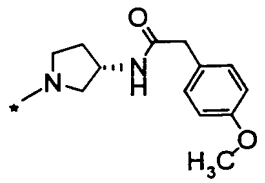
LRMS: m/z 464 (MH^+)

EXAMPLE 11-12

The compounds of the following tabulated examples with the general formula:

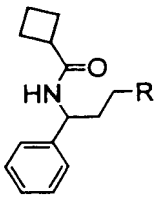


were prepared using a similar method to example 10 from the title compound of preparation 26 and the corresponding amines.

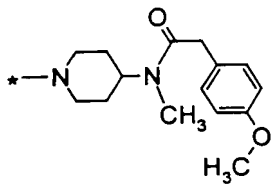
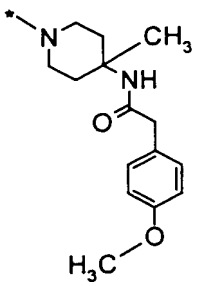
EX. NO.	R	YIELD (%)	CHARACTERIZATION DATA
11		37	Found: C, 72.00; H, 7.87; N, 9.30% $C_{27}H_{35}N_3O_3$; requires C, 72.13; H, 7.85; N 9.35% ^1H NMR (300 MHz, $CDCl_3$): δ [ppm] 1.80-2.38 (12H, m), 2.37 (2H, m), 2.58 (1H, t), 2.78 (1H, m), 2.92 (1H, m), 3.51 (2H, s), 3.78 (3H, s), 4.38 (1H, m), 5.18 (1H, m), 5.95 (1H, br d), 6.57 (1H, br d), 6.85 (2H, d), 7.20 (7H, m) LRMS: m/z 450.6 (MH^+) Melting point [°C]: 175-176 (ether) $[\alpha]_D$ -33.5° (c = 0.27, MeOH)
12		8	Found: C, 71.97; H, 7.87; N, 9.33% $C_{27}H_{35}N_3O_3$; requires C, 72.13; H, 7.85; N 9.35% ^1H NMR (300 MHz, $CDCl_3$): δ [ppm] 1.40-1.55 (1H, m), 1.80-2.01 (4H, m), 2.03-2.55 (9H, m), 2.60-2.75 (2H, m), 2.92 (1H, m), 3.43 (2H, s), 3.80 (3H, s), 4.40 (1H, m), 5.05 (1H, m), 5.80 (1H, br d), 6.60 (1H, br d), 6.85 (2H, d), 7.10-7.35 (7H, m) LRMS: m/z 449.3 (MH^+) Melting point [°C]: 145-146 (ether) $[\alpha]_D$ -62.5° (c = 0.17, MeOH)

EXAMPLE 13-14

The compounds of the following tabulated examples with the general formula:

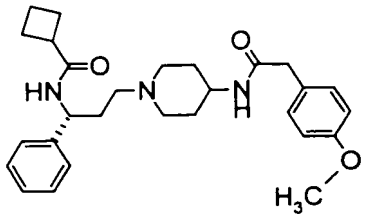


were prepared using a similar method to example 10 from the title compound of preparation 21 and the corresponding amine.

EX. NO.	R	YIELD (%)	CHARACTERIZATION DATA
13		55	Found: C, 70.30; H, 8.56; N, 8.61% $C_{29}H_{39}N_3O_3;H_2O$ requires C, 70.27; H, 8.34; N, 8.48% ^1H NMR (400 MHz, $CDCl_3$): δ [ppm] 1.25-1.39 (1H, m), 1.58-2.20 (13H, m), 2.20-2.38 (3H, m), 2.80-2.95 (3H, m), 2.95-3.08 (2H, m), 3.58-3.72 (2H, m), 3.78 (3H, s), 4.45-4.58 (1H, m), 5.05-5.15 (1H, m), 6.82-6.90 (2H, m), 7.12-7.35 (7H, m) LRMS: m/z 478.2 (MH^+)
14		26	Found: C, 71.36; H, 8.19; N, 8.51% $C_{29}H_{39}N_3O_3;0.6H_2O$; requires C, 71.31; H, 8.30; N, 8.60% ^1H NMR (400 MHz, $CDCl_3$): δ [ppm] 1.49-1.62 (8H, m), 1.72-2.02 (8H, m), 2.10-2.20 (2H, m), 2.20-2.35 (2H, m), 2.40-2.50 (1H, m), 2.52-2.62 (1H, m), 3.42 (2H, s), 3.79 (3H, s), 5.02-5.12 (1H, m), 6.82-6.91 (2H, m), 7.15-7.37 (6H, m), 7.41-7.49 (1H, m) LRMS: m/z 478.2 (MH^+)

EXAMPLE 15

N-[(1*R*)-3-(4-{[2-(4-Methoxyphenyl)acetyl]amino}-1-piperidinyl)-1-phenylpropyl]cyclobutanecarboxamide



The title compound was prepared using a similar method to example 2 from the title compound of preparation 61 and cyclobutanecarbonyl chloride, 56%.

Found: C, 69.80; H, 7.80; N, 8.72%

$C_{28}H_{37}N_3O_3;0.1H_2O$ requires C, 69.83; H, 8.16; N, 8.72%

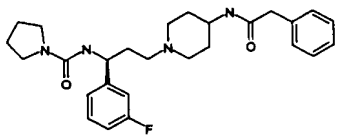
^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] 1.23-1.40 (2H, m), 1.74-2.32 (15H, m), 2.60-2.70 (1H, m), 2.70-2.80 (1H, m), 2.97 (1H, m), 3.48 (2H, s), 3.71-3.84 (1H, m), 3.81 (3H, s), 5.08 (1H, m), 5.21 (1H, d), 6.89 (2H, d), 7.13-7.23 (5H, m), 7.23-7.32 (2H, m)

LRMS: m/z 464 (MH^+)

$[\alpha]_D$ +37.8° (c = 0.18, MeOH)

EXAMPLE 16

N-((1*S*)-1-(3-Fluorophenyl)-3-{4-[(2-phenylacetyl)amino]-1-piperidinyl}propyl)-1-pyrrolidinecarboxamide



The title compound of preparaiton 56 (140mg, 0.30mmol) was stirred for 30 minutes in a 5ml mixture of trifluoroacetic acid:dichloromethane. The solvents were removed under reduced pressure and basified with saturated sodium carbonate solution before extracting with dichloromethane (x3). The organic layers were combined and dried ($MgSO_4$), filtered and evaporated under reduced pressure. The residue (100mg, 0.27mmol) in 1ml of dichloromethane was added to a mixture of 1,1'-carbonyldiimidazole (44mg, 2.71mmol) and imidazole (18mg, 2.71mmol) in 1ml of dichloromethane at 0°C. The mixture was stirred for 15 minutes and then warmed slowly to room temperature over 1 hour. A solution of pyrrolidine (26mg, 2.71mmol) in 1ml of dichloromethane was added and the mixture stirred for 18 hours. The mixture was diluted with dichloromethane and washed with water. The organic layer was dried ($MgSO_4$), filtered and evaporated under reduced pressure. the residue was purified by column chromatography on silica gel using an eluent of dichloromethane:methanol:0.88 ammonia (95:5:0.5) to afford the title compound as a colourless glass, 39mg.

Found: C, 68.46; H, 7.68; N, 11.85%

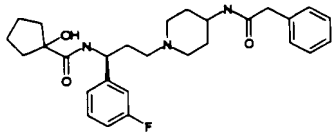
$C_{27}H_{35}FN_4O_2;0.5\ H_2O$ requires C, 68.19; H, 7.63; N, 11.78%

^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] 1.20-1.37 (2H, m), 1.71-1.81 (1H, m), 1.81-1.94 (6H, m), 1.94-2.11 (3H ,m), 2.19-2.34 (2H, m), 2.60-2.71 (1H, m), 2.71-2.80 (1H, m), 3.26-3.37 (4H, m), 3.55 (2H, s), 3.73-3.82 (1H, m), 4.94-5.00 (1H, m), 5.13-5.23 (1H, m), 6.13-6.20 (1H, m), 6.84-6.90 (1H, m), 6.90-6.97 (1H, m), 7.00-7.03 (1H, m), 7.20-7.26 (3H, m), 7.26-7.32 (1H, m), 7.32-7.40 (2H, m)

LRMS: m/z 467.1 (MH^+)

EXAMPLE 17

N-((1*S*)-1-(3-Fluorophenyl)-3-{4-[(2-phenylacetyl)amino]-1-piperidinyl}propyl)-1-hydroxycyclopentanecarboxamide



The title compound of preparation 56 (140mg, 0.30mmol) was stirred for 30 minutes in a mixture of trifluoroacetic acid:dichloromethane (5ml, 1:1). The solvents were removed under reduced pressure and basified with saturated sodium carbonate solution before extracting with dichloromethane (x3). The organic layers were combined and dried ($MgSO_4$), filtered and evaporated under reduced pressure. The residue (100mg, 2.71mmol), 1-hydroxycyclopentanecarboxylic acid (39mg, 2.98mmol), 1-hydroxybenzotriazole monohydrate (44mg, 3.25mmol), 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (67mg, 3.52mmol) and triethylamine (0.05ml, 3.52mmol) were stirred together for 18 hours in dichloromethane (6ml). The solvent was removed under reduced pressure and the residue dissolved in ethyl acetate, then washed with water (x2). The organic layer was dried ($MgSO_4$), filtered and evaporated under reduced pressure. the residue was purified by column chromatography on silica gel using an eluent of dichlromethane:methanol:0.88 ammonia (95:5:0.5) to afford the title compound as a white foam, 93mg.

Found: C, 68.69; H, 7.60; N, 8.55%

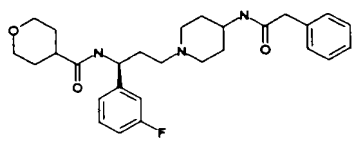
$C_{28}H_{37}FN_3O_3$;0.4 H_2O requires C, 68.66; H, 7.78; N, 8.58%

^1H NMR (400 MHz, $CDCl_3$): δ [ppm] 1.35-1.48 (2H, m), 1.65-1.94 (7H, m), 1.94-2.24 (7H, m), 2.24-2.35 (2H, m), 2.60-2.69 (1H, m), 2.76-2.85 (1H, m), 3.56 (2H, s), 3.68-3.81 (1H, m), 5.05-5.11 (1H, m), 5.20-5.27 (1H, m), 6.87-6.94 (2H, m), 6.97-7.01 (1H, m), 7.23-7.40 (7H, m), 8.53-8.61 (1H, m)

LRMS: m/z 482.1 (MH^+)

EXAMPLE 18

N-((1*S*)-1-(3-Fluorophenyl)-3-{4-[(2-phenylacetyl)amino]-1-piperidinyl}propyl)tetrahydro-2*H*-pyran-4-carboxamide



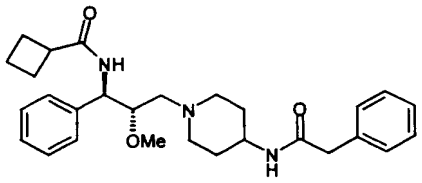
The title compound was prepared using a similar procedure to that described in example 17 from tetrahydro-2*H*-pyran-4-carboxylic acid and purifying by recrystallisation from ethyl acetate in a 51% yield, 66mg.

^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] 1.21-1.40 (3H, m), 1.66-1.84 (5H, m), 1.84-2.16 (5H, m), 2.16-2.24 (1H, m), 2.24-2.39 (2H, m), 2.63-2.74 (1H, m), 2.77-2.87 (1H, m), 3.27-3.39 (2H, m), 3.55 (2H, s), 3.74-3.84 (1H, m), 3.90-4.00 (2H, m), 5.02-5.08 (1H, m), 5.20-5.27 (1H, m), 6.82-6.94 (2H, m), 6.94-6.99 (1H, m), 7.21-7.39 (5H, m), 7.97-8.05 (1H, m)

LRMS: m/z 482.3 (MH^+)

EXAMPLE 19

N-((1*R*,2*S*)-2-Methoxy-1-phenyl-3-{4-[(2-phenylacetyl)amino]-1-piperidinyl}propyl)cyclobutanecarboxamide



Diisobutylaluminium hydride (3.47ml of a 1.0M solution in dichloromethane, 3.47mmol) was added dropwise to a solution of the title compound of preparation 51 (460mg, 1.58mmol) in dichloromethane (20ml) at -78°C. The reaction mixture was stirred at this temperature for 1 hour, then methanol (2ml) pre-cooled to -78°C was added. The mixture was warmed to room temperature and washed with 2M hydrochloric acid, water and brine, dried ($MgSO_4$), filtered and the solvent evaporated under reduced pressure to afford the aldehyde as a clear oil, 400mg. This oil (400mg, 1.53mmol), the title compound of preparation 2 (334mg, 1.53mmol) and sodium triacetoxyborohydride (483mg, 2.28mmol) were stirred together for 3 hours at room temperature in dichloromethane:acetic acid (30ml, 10%). The solvents were evaporated under reduced pressure and the residue dissolved in ethyl acetate and washed with saturated aqueous sodium carbonate solution then water. The organic layer was dried ($MgSO_4$), filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography on silica gel using dichloromethane:methanol:0.88 ammonia (95:5:0.5) as eluant to afford the title compound as a white solid, 117mg.

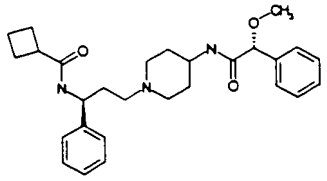
^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] 1.31 (3H, m), 1.81 (4H, m), 2.05-2.18 (7H, m), 2.61 (1H, d), 2.76 (1H, d), 3.05 (1H, m), 3.25 (3H, s), 3.48 (1H, m), 3.58 (2H, m), 3.79 (1H, m), 5.23 (2H, m), 6.78 (1H, d), 7.18-7.38 (10H, m)

LRMS: m/z 464.1 (MH^+)

$[\alpha]_D$: -28.1 (c = 5.10, methanol)

EXAMPLE 20

N-[(1*S*)-3-(4-{[(2*R*)-2-Methoxy-2-phenylethanoyl]amino}-1-piperidinyl)-1-phenylpropyl]cyclobutanecarboxamide



A solution of the title compound of preparation 72 (100mg, 0.32mmol) in dichloromethane (2.5 ml) was added to a solution of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (60mg, 0.32mmol), (*R*)-α-methoxyphenyl acetic acid (53mg, 0.32mmol), 1-hydroxybenzotriazole hydrate (50mg, 0.33mmol), and diisopropylethylamine (55μl, 0.32 mmol) in dichloromethane (2.5 ml). The reaction mixture was stirred for 30 hours, then diluted with dichloromethane and washed with 10% sodium carbonate. The layers were separated and the aqueous layer was extracted with dichloromethane (2x 25ml). The organic layers were combined, washed with brine, dried ($MgSO_4$), filtered and the solvent was evaporated. The crude product was purified by column chromatography on silica gel using dichloromethane:methanol:0.88 ammonia (95:5:0.5) as the eluant to yield the title product, 60 mg.

Found: C, 70.25; H, 8.18; N, 8.49%

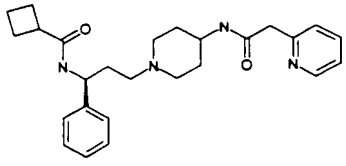
$C_{28}H_{37}N_3O_3;0.9H_2O$ requires C, 70.09; H, 8.15; N, 8.76%

^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] 1.46 (2H, m), 1.76-2.41 (15H, m), 2.78 (1H, bm), 2.84 (1H, bm), 3.02 (1H, m), 3.36 (3H, s), 3.80 (1H, bm), 4.59 (1H, s), 5.12 (1H, m), 6.64 (1H, d), 7.16-7.42 (10H, m)

LRMS: m/z 464 (MH^+)

EXAMPLE 21

N-[(1*S*)-1-Phenyl-3-(4-{[2-(2-pyridinyl)acetyl]amino}-1-piperidinyl)propyl]cyclobutanecarboxamide



The title compound was prepared using a similar method to that described for Example 20 from the title compound of preparation 72 and 2-pyridyl acetic acid hydrochloride, 35%.

Found: C, 68.08; H, 8.05; N, 12.20%

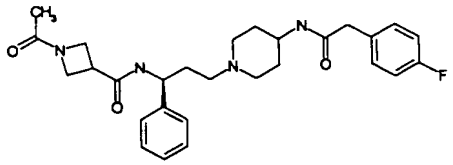
$C_{26}H_{34}N_4O_2;1.3H_2O$ requires C, 68.08; H, 8.06; N, 12.21%

^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] 1.46-2.41 (18H, m), 2.67 (1H, bm), 2.81 (1H, bm), 3.03 (1H, m), 3.72 (2H, s), 3.83 (1H, bm), 5.10 (1H, m), 7.14-7.32 (5H, m), 7.40 (1H, m), 7.52 (1H, m), 7.67 (1H, t), 8.54 (1H, d)

LRMS: m/z 436 (MH^+)

EXAMPLE 22

1-Acetyl-*N*-[(1*S*)-3-(4-{[2-(4-fluorophenyl)acetyl]amino}-1-piperidinyl)-1-phenylpropyl]-3-azetidinecarboxamide



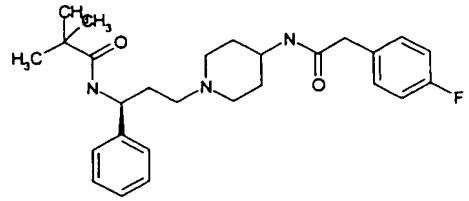
The title compound of preparation 70 (75mg, 0.2mmol), 1-acetyl-3-azetidinecarboxylic acid (29mg, 0.2mmol), 1-hydroxybenzotriazole monohydrate (30mg, 0.22mmol), 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (47mg, 0.24mmol) and triethylamine (0.034ml, 0.24mmol) were stirred together for 4 hours in 5ml of dichloromethane. The mixture was evaporated under reduced pressure and the residue dissolved in ethyl acetate before washing with water, saturated sodium carbonate solution then water. The organic layer was dried ($MgSO_4$), filtered and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel using an eluant of dichloromethane:methanol:0.88 ammonia (95:5:0.5) to afford the title compound as a white foam, 45mg.

^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] 1.20-1.52 (3H, m), 1.68-1.81 (1H, m), 1.18-1.85 (3H, m), 1.85-2.00 (2H ,m) ,2.00-2.24 (3H, m), 2.29-2.45 (1H, m), 2.71-2.80 (1H, m), 2.90-3.03 (1H, m), 3.23-3.35 (1H, m), 3.50 (2H, s), 3.74-3.87 (1H, m), 4.13-4.35 (4H, m), 5.06-5.13 & 5.13-5.21 (1H, m), 5.94-6.00 & 6.13-6.20 (1H, m), 6.98-7.03 (2H, m), 7.13-7.19 (2H, m), 7.19-7.34 (5H, m), 8.53-8.60 & 8.61-8.68 (1H, m)

LRMS: m/z 495.1 (MH^+)

EXAMPLE 23

N-[(1*S*)-3-(4-{[2-(4-Fluorophenyl)acetyl]amino}-1-piperidinyl)-1-phenylpropyl]-2,2-dimethylpropanamide



The title compound was prepared using a similar method to that described for example 1 from the title compound of preparation 70 and trimethylacetyl chloride using diisopropylethylamine as base, 43%.

Found: C, 66.74; H, 7.60; N, 8.59%

$C_{27}H_{36}FN_3O_2$;1.7H_2O requires C, 66.97; H, 8.20; N, 8.68%

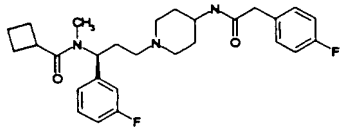
^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] 1.19 (9H, s), 1.36 (2H, m), 1.72-1.84 (1H, m), 1.90 (2H, bm), 1.95-2.14 (3H, m), 2.14-2.24 (1H, m), 2.28-2.38 (1H, m), 2.72 (1H, m), 2.82 (1H, m), 2.97 (1H, m), 3.52 (2H, s), 3.78 (1H, m), 5.04 (1H, m), 5.11 (1H, d), 7.04 (2H, t), 7.13-7.14-7.34 (6H, m), 7.46 (1H, m)

LRMS: m/z 454 (MH^+)

Melting point [°C]: 158-159

EXAMPLE 24 UK-386739

N-[(1*S*)-1-(3-Fluorophenyl)-3-(4-{[2-(4-fluorophenyl)acetyl]amino}-1-piperidinyl)propyl]-*N*-methylcyclobutanecarboxamide



To a mixture of the title compound of preparation 67 (100mg, 0.25mmol) and triethylamine (0.04ml, 0.28mmol) in 5ml of dichloromethane was added cyclobutylcarbonyl chloride (0.03ml, 0.25mmol). The mixture was evaporated under reduced pressure and the residue dissolved in ethyl acetate before washing with saturated sodium carbonate solution then water. The organic layer was dried ($MgSO_4$), filtered and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel using an eluent of dichloromethane:methanol:0.88 ammonia (98:2:0.25) to afford the title compound as a gum, 20mg.

^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] 1.26-1.40 (2H, m), 1.80-2.00 (5H, m), 2.00-2.40 (9H, m), 2.56 & 2.68 (3H, s), 2.69-2.80 (2H, m), 3.20-3.29 (1H, m), 3.50 (2H, s), 3.71-3.82 (1H, m), 5.13-5.23 (1H, m), 5.87-5.94 (1H, m), 6.84-7.00 (3H, m), 7.00-7.06 (2H, m), 7.20-7.32 (3H, m)

LRMS: m/z 484.2 (MH^+)

CLAIMS

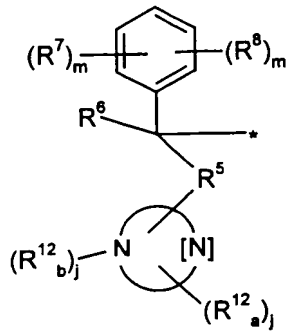
1. A compound of Formula (I);

$$[R_{egion\ \alpha}] - [R_{egion\ \beta}] - [R_{egion\ \gamma}] - [R_{egion\ \delta}] \qquad (I)$$

wherein **$[R_{egion\ \alpha}]$** is selected from the group consisting of:

-A. Aryl heterocyclyl substituent components comprising:

--1. hetero-phenylmethylene moieties of partial Formula (1.0.0):



(1.0.0)

---wherein: the symbol " * " indicates the point of attachment of the moiety of partial Formula (1.0.0) to $R_{egion\ \beta}$, as hereinafter defined;

---R^5 is a member selected from the group consisting of a direct bond; -O-; -C(=O)-; $-NR^4-$; and $-S(=O)_p-$; where:

----R^4 is hydrogen or (C_1-C_2)alkyl;

---R^6 is a member selected from the group consisting of hydrogen; (C_1-C_2)alkyl; (C_1-C_2)alkoxy; -CN; -OH; and $-C(=O)NH_2$;

---j is an integer selected from 0, 1, and 2;

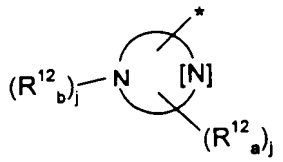
---m is an integer selected from 0, 1, and 2;

---R^7 and R^8 are each a member selected from the group consisting of -F; -Cl; $-CO_2R^4$; -OH; -CN; $-CONR^4{}_aR^4{}_b$; $-NR^4{}_aR^4{}_b-$; $-NR^4{}_aC(=O)R^4{}_b$; $-NR^4{}_aC(=O)OR^4{}_b$; $-NR^4{}_aS(=O)_pR^4{}_b$; $-S(=O)_pNR^4{}_aR^4{}_b$; (C_1-C_4)alkyl, and (C_1-C_4)alkoxy wherein said alkyl and alkoxy are each substituted with 0 to 3 substituents independently selected from F and Cl; (C_1-C_2)alkoxycarbonyl; (C_1-C_2)alkylcarbonyl; and (C_1-C_2)alkylcarbonyloxy; where:

----p is an integer selected from 0, 1, and 2;

---R^4_a and R^4_b are each independently selected from hydrogen and $(C_1\text{-}C_2)$alkyl;

---the moiety represented by partial Formula (1.0.1):



(1.0.1)

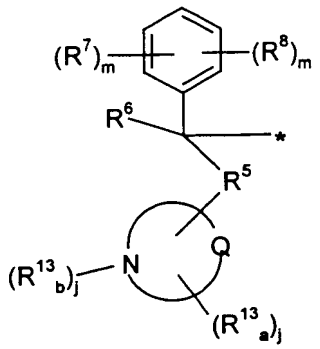
in partial Formula (1.0.0) represents a monocyclic heterocyclic group, or a bicyclic benzo-fused ring system containing said heterocyclic group wherein said heterocyclic group contains a total of 5- or 6- members of which one or two of said members is nitrogen, the presence of the optional second nitrogen atom being represented by: "[N]"; wherein said heterocyclic group or ring system are selected from the group consisting of pyrrolyl; pyrazolyl; imidazolyl; pyridinyl; pyrazinyl; pyrimidinyl; pyridazinyl; piperazinyl; indolyl; indazolinyl; benzimidazolyl; quinolinyl; *iso*-quinolinyl; and quinazolinyl; wherein:

---R^{12}_a is a member selected from the group consisting of hydrogen; F; Cl; $-CO_2R^4$; oxo; -OH; CN; NH_2; $NH(C_1\text{-}C_2)$alkyl; $N(C_1\text{-}C_2)_2$dialkyl; $-CF_3$; $(C_1\text{-}C_4)$alkyl; $(C_2\text{-}C_4)$alkenyl; $(C_1\text{-}C_4)$alkoxy; $(C_3\text{-}C_7)$cycloalkyl; and phenyl; wherein said alkyl, alkenyl, alkoxy, cycloalkyl and phenyl are substituted with 0 to 2 substituents R^9 where:

----R^9 is a member independently selected from the group consisting of F; Cl; $-CO_2R^4$; -OH; cyano; $-CONR^4_aR^4_b$; $-NR^4_aR^4_b$-; $-NR^4_aC(=O)R^4_b$; $-NR^4_aC(=O)OR^4_b$; $-NR^4_aS(=O)_pR^4_b$; $-S(=O)_pNR^4_aR^4_b$; $(C_1\text{-}C_4)$alkyl including dimethyl, and $(C_1\text{-}C_4)$alkoxy wherein said alkyl and alkoxy are each independently substituted with 0 to 3 substituents independently selected from F and Cl; $(C_1\text{-}C_2)$alkoxycarbonyl; $(C_1\text{-}C_2)$alkylcarbonyl; and $(C_1\text{-}C_2)$alkylcarbonyloxy; and

----R^{12}_b is absent or is a member selected from the group consisting of hydrogen; $(C_1\text{-}C_4)$alkyl; $(C_2\text{-}C_4)$alkenyl; $(C_1\text{-}C_2)$alkoxy; $(C_3\text{-}C_7)$cycloalkyl; and phenyl; wherein said alkyl, alkenyl, alkoxy, cycloalkyl and phenyl are substituted with 0 to 2 substituents R^9 wherein R^9 has the same meaning as above, except that it is selected independently selected therefrom; and

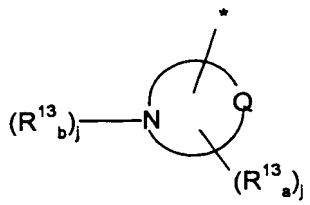
2. hetero-phenylmethylene moieties of partial Formula (1.1.0):



(1.1.0)

---wherein: the symbol " * "; R^5; R^6; R^7; R^8; j and m are as defined further above, except that all of the above-recited substituents are selected independently of their selection above;

---the moiety represented by partial Formula (1.1.1):



(1.1.1)

in partial Formula (1.1.0) represents:

----a. a monocyclic heterocyclic group containing a total of 5 or 6 members of which one said member is nitrogen and Q is selected from O and S where said S may optionally be in the sulfonate form, $-S(=O)_2$; wherein said heterocyclic group is selected from the group consisting of oxazolyl; oxazolidinyl; *iso*xazolyl; thiazolyl; thiazolidinyl; *iso*-thiazolyl; morpholinyl; and thiomorpholinyl; or

----b. a monocyclic heterocyclic group containing a total of 5- or 6- member s of which two said members are nitrogen and a third or fourth said member is independently selected from N, O, and S where said S may optionally be in the sulfonate form, $-S(=O)_2$; wherein said heterocyclic group is selected from the group consisting of triazolyl; triazinyl; tetrazolyl; oxadiazolyl; thiadiazolyl; and

----$R^{13}{}_a$ is selected from the group consisting of hydrogen; F; Cl; $-CO_2R^4$; oxo; -OH; CN; NH_2; $NH(C_1\text{-}C_2)$alkyl; $N(C_1\text{-}C_2)_2$dialkyl; $-CF_3$; $(C_1\text{-}C_4)$alkyl; $(C_2\text{-}C_4)$alkenyl; $(C_1\text{-}C_2)$alkoxy; $(C_3\text{-}C_7)$cycloalkyl; and phenyl; wherein said alkyl, alkenyl, alkoxy, cycloalkyl and phenyl are substituted with 0 to 2 substituents R^{11} where:

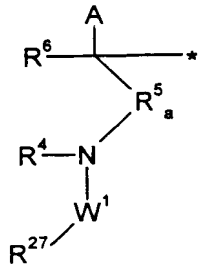
----R^{11} is a member selected from the group consisting of F; Cl; $-CO_2R^4$; -OH; -CN; $-CONR^4{}_aR^4{}_b$; $-NR^4{}_aR^4{}_b$; $-NR^4{}_aC(=O)R^4{}_b$; $-NR^4{}_aC(=O)OR^4{}_b$; $-NR^4{}_aS(=O)_pR^4{}_b$;

$-S(=O)_pNR^4{}_aR^4{}_b$; $(C_1\text{-}C_4)$alkyl including dimethyl, and $(C_1\text{-}C_4)$alkoxy wherein said alkyl and alkoxy are each independently substituted with 0 to 3 substituents independently selected from F and Cl; $(C_1\text{-}C_2)$alkoxycarbonyl; $(C_1\text{-}C_2)$alkylcarbonyl; and $(C_1\text{-}C_2)$alkylcarbonyloxy; and

---$R^{13}{}_b$ is a member selected from the group consisting of hydrogen; $(C_1\text{-}C_4)$alkyl; $(C_2\text{-}C_4)$alkenyl; $(C_1\text{-}C_2)$alkoxy; $(C_3\text{-}C_7)$cycloalkyl; $C(=O)(C_1\text{-}C_4)$alkyl; $S(=O)_2(C_1\text{-}C_4)$alkyl; and phenyl; wherein said alkyl, alkenyl, alkoxy, cycloalkyl and phenyl are substituted with 0 to 2 substituents R^{11} wherein R^{11} has the same meaning as in above, except that it is selected independently;

-B. a (substituted)-amido-aryl or -heterocyclyl moiety selected from the group consisting of

--1. alkyl-, alkenyl-, and alkynyl-substituted-amido-aryl moieties of partial Formula (2.0.0):

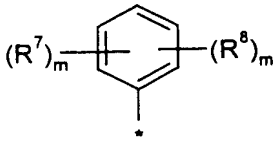


(2.0.0)

---wherein: the symbol " * "; R^4 and R^6; are as defined above, except that all of the above-recited substituents are selected independently of their selection above;

---A is a member selected from the group consisting of:

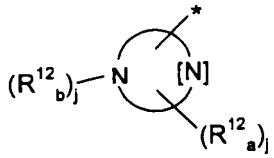
----1. the moiety of partial Formula (2.0.3)



(2.0.3)

-----wherein: the symbol R^7; R^8 and m are as defined above, except that all of the above-recited substituents are selected independently of their selection above; and the symbol: " * " indicates the point of attachment of the moiety A to the, remaining portions of partial Formula (2.0.0);

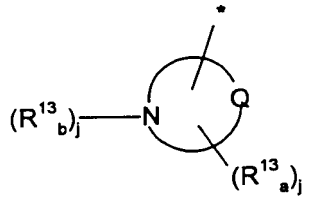
----2. the moiety of partial Formula (2.0.4)



(2.0.4)

which represents a monocyclic heterocyclic group, selected from the group consisting of pyrrolyl; pyrazolyl; imidazolyl; pyridinyl; pyrazinyl; pyrimidinyl; wherein: the symbol $R^{12}{}_a$ and $R^{12}{}_b$ are as defined above, except that all of the above-recited substituents are selected independently of their selection above; and the symbol: " * " indicates the point of attachment of the moiety A to the other, remaining portions of partial Formula (2.0.0);

---3. the moiety of partial Formula (2.0.5)



(2.0.5)

which represents

----a. a monocyclic heteroaromatic group containing a total of 5- members of which one said member is nitrogen and Q is selected from O and S where said S may optionally be in the sulfonate form, $-S(=O)_2$; selected from the group consisting of oxazolyl; *iso*xazolyl; thiazolyl; and *iso*-thiazolyl; or

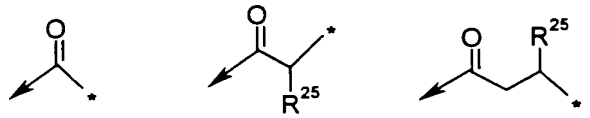
----b. a monocyclic heterocyclic group containing a total of 5- or 6- members of which two said members are nitrogen and a third or fourth said member is independently selected from N, O, and S where said S may optionally be in the sulfonate form, $-S(=O)_2$; selected from the group consisting of triazolyl; triazinyl; tetrazolyl; oxadiazolyl; and thiadiazolyl; and

-----wherein: the $R^{13}{}_a$, $R^{13}{}_b$ and j are as defined above, except that all of the above-recited substituents are selected independently of their selection above; and the symbol: " * " indicates the point of attachment of the moiety A to the other, remaining portions of partial Formula (2.0.2);

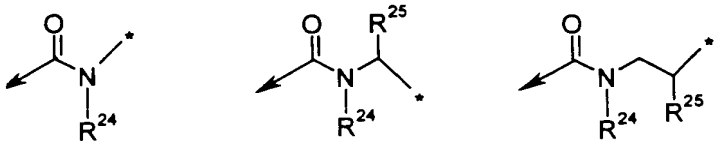
---$R^5{}_a$ is a member selected from the group consisting of a direct bond; -C(=O)-; and $-S(=O)_2$-;

---W^1 is (1.) a direct bond; (2.) in the case where $R^5{}_a$ is -C(=O)- or $-S(=O)_2$, W^1 is a direct bond or $-(C_1\text{-}C_3)$alkylene- wherein any single carbon atom thereof is substituted by 0 to 2 substituents R^{23} where R^{23} is a member selected from the group consisting of -F; -Cl;

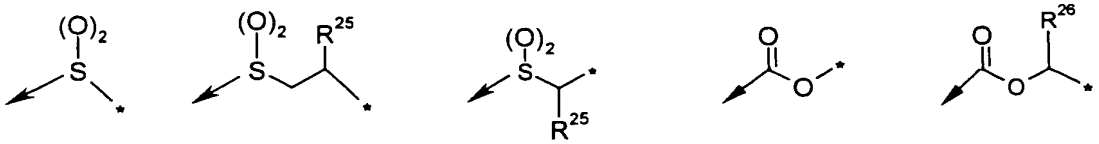
$-CO_2R^4$; -OH; -CN; (C_1-C_4)alkoxy; (C_3-C_7)cycloalkyl; and phenyl; wherein said alkoxy, cycloalkyl, and phenyl are substituted with 0 to 2 substituents R^{11}, wherein said R^{11} is as defined above, except that all of the above-recited substituents are selected independently of their selection above; or (3.) is a member independently selected from the group consisting of the moieties of partial Formulas (2.0.6) through (2.0.16), inclusive:



(2.0.6) (2.0.7) (2.0.8)



(2.0.9) (2.0.10) (2.0.11)



(2.0.12) (2.0.13) (2.0.14) (2.0.15) (2.0.16)

—wherein: the symbol: "→" indicates the point of attachment of the moiety W^1 to the nitrogen atom in partial Formula (2.0.0), and the symbol: " * " indicates the point of attachment of the moiety W^1 to the other, remaining portions of partial Formula (2.0.0); and R^4 is as defined further above, but selected on an independent basis;

———R^{24} is selected from the group consisting of hydrogen and (C_1-C_4)alkyl; and

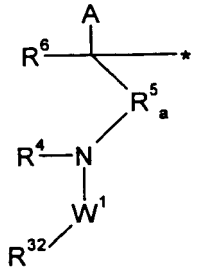
—R^{25} and R^{26} are each selected from the group consisting of -OH; (C_1-C_2)alkyl substituted by 0 to 3 substituents selected from F; and OH; and (C_1-C_2)alkoxy; and

—R^{27} is selected from the group consisting of (C_1-C_6)alkyl; (C_2-C_6)alkenyl; and (C_2-C_6)alkynyl; wherein said alkyl, alkenyl, and alkynyl groups comprising R^{27} are substituted with 0 to 3 substituents R^{28} where:

—R^{28} is selected from the group consisting of phenyl; F or Cl; oxo; hydroxy; (C_1-C_2)alkyl; (C_1-C_3)alkoxy; $-C(=O)OR^{29}$; $-C(=O)(C_1-C_4)$alkyl; $-S(=O)_2(C_1-C_4)$alkyl; $-C(=O)NR^{29}R^{30}$; $-NR^{29}R^{30}$; $-NR^{29}C(=O)R^{30}$; $-NR^{29}C(=O)OR^{30}$; $-NR^{29}S(=O)_pR^{30}$; and $-S(=O)_2NR^{29}R^{30}$, where:

——R^{29} and R^{30} are each a member independently selected from the group consisting of hydrogen and (C_1-C_4)alkyl substituted by 0 to 3 substituents selected from the group consisting of F and Cl;

–2. cycloalkyl-substituted-amido-aryl moieties of partial Formula (2.1.0):

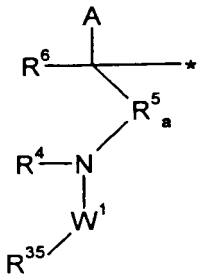


(2.1.0)

—wherein: A; W^1; the symbol " * "; R^4; $R^5{}_a$; and R^6 have the same meaning as set out above, except that all of the above-recited substituents are selected independently of their selection above; and

—R^{32} is a member selected from the group consisting of -$(CH_2)_n$-(C_3-C_7)cycloalkyl, where n is an integer selected from 0, 1, and 2; in the event n is 0, then the α-carbon atom of said (C_3-C_7)cycloalkyl is substituted by 0 or 1 (C_1-C_4)alkyl or phenyl, where said alkyl or phenyl are substituted by 0, 1, or 2 of CH_3, OCH_3, OH or NH_2; and in the event that n is 1 or 2, the resulting methylene or ethylene is substituted by 0 or 1 of F; NH_2; $N(CH_3)_2$; OH; OCH_3; (C_1-C_4)alkyl; or phenyl; where said alkyl and phenyl are substituted by 0, 1, or 2 of CH_3, OCH_3, OH, and NH_2; and further wherein said (C_3-C_7)cycloalkyl is substituted by 0 to 3 substituents R^{28} where R^{28} is as defined further above, but selected independently

–3. aryl and heterocyclic-substituted-amido-aryl moieties of partial Formula (2.2.0):

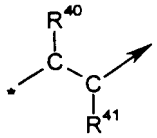


(2.2.0)

—wherein: A; W^1; the symbol: " * "; R^4; $R^5{}_a$; and R^6 have the same meaning as set out above, except that all of the above-recited substituents are selected independently of their selection above; and

—R^{35} is selected from the group consisting of phenyl; furyl; tetrahydrofuranyl; tetrahydropyranyl; oxetanyl; thienyl; pyrrolyl; pyrrolidinyl; oxazolyl; isoxazolyl; thiazolyl; isothiazolyl; imidazolyl; pyrazolyl; oxadiazolyl; thiadiazolyl; triazolyl; pyridyl; pyrazinyl; pyridazinyl; piperazinyl; pyrimidinyl; pyranyl; azetidinyl; morpholinyl; parathiazinyl; indolyl; indolinyl; benzo[*b*]furanyl; 2;3-dihydrobenzofuranyl; benzothienyl; 1H-indazolyl; benzimidazolyl; benzoxazolyl; benzisoxazolyl; benzthiazolyl; quinolinyl; isoquinolinyl; phthalazinyl; quinazolinyl; and quinoxalinyl; wherein (1.) said group R^{35} may be substituted upon any one or more carbon atoms thereof by 0 to 3 substituents R^{28} where R^{28} is as defined above, except that it is selected independently; (2.) said group R^{35} is substituted with respect to any one or more nitrogen atoms thereof that is not a point of attachment of said aryl or heterocyclic moiety, by 0 to 3 substituents $R^{13}{}_b$ where $R^{13}{}_b$ is as defined above, except that it is selected independently; and (3.) said group R^{35} with respect to any sulfur atom thereof that is not a point of attachment of said heterocyclic moiety, is substituted by 0 or 2 oxygen atoms;

[R_{eglon} β] is an alkyl bridging element of partial Formula (3.0.0):



(3.0.0)

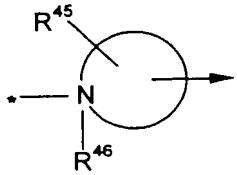
wherein:

—" * " is a symbol which represents the point of attachment of the moiety of partial Formula (3.0.0) to R_{eglon} α;

—"→" is a symbol which represents the point of attachment of the moiety of partial Formula (3.0.0) to R_{eglon} γ;

—R^{40} and R^{41} are both selected from the group consisting of hydrogen; (C_1-C_2) alkyl including dimethyl; hydroxy; and (C_1-C_3) alkoxy;

[R_{eglon} γ] is an aza-monocyclic moiety of partial Formula (4.0.0):



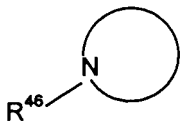
(4.0.0)

—wherein:

—" * " is a symbol which represents the point of attachment of the moiety of partial Formula (4.0.0) to R_{egion} β of the compound of Formula (I);

—"*→*" is a symbol representing a covalent bond attaching any carbon atom of said aza-monocyclic moiety of partial Formula (4.0.0) to R_{egion} δ;

—the moiety of partial Formula (4.0.1):



(4.0.1)

in partial Formula (4.0.0) represents a monocyclic heterocyclic group containing a total of from 4- to 7-members of which one said member is nitrogen, wherein said heterocyclic group is a member independently selected from the group consisting essentially of azetidinyl; pyrrolidinyl; piperidinyl; and azepinyl;

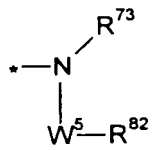
—R^{45} is absent or is a member independently selected from the group consisting essentially of $(C_1\text{-}C_4)$alkyl including dimethyl; $(C_3\text{-}C_6)$cycloalkyl; $(C_1\text{-}C_4)$alkoxy; CF_3; $-CO_2R^4$ where R^4 is as defined further above; oxo; -OH; cyano; $-C(=O)NR^4{}_aR^4{}_b$; $-NR^4{}_aR^4{}_b$; $-NR^4{}_aC(=O)R^4{}_b$; $-NR^4{}_aC(=O)OR^4{}_b$; $-NR^4{}_aS(=O)_pR^4{}_b$; $-S(=O)_pNR^4{}_aR^4{}_b$; $(C_1\text{-}C_2)$alkoxycarbonyl; $(C_1\text{-}C_2)$alkylcarbonyl; $(C_1\text{-}C_2)$alkylcarbonyloxy and $(C_1\text{-}C_2)$alkoxy$(C_1\text{-}C_2)$alkyl; it being understood that in the moiety of partial Formula (4.0.0) R^{45} is a substituent attached to a single carbon atom thereof; where:

—$R^4{}_a$ and $R^4{}_b$ are each independently selected from hydrogen and $(C_1\text{-}C_2)$alkyl;

—R^{46} is absent or is a member independently selected from the group consisting essentially of hydrogen; and $(C_1\text{-}C_4)$alkyl substituted by 0 or 1 substituent independently selected from $(C_1\text{-}C_2)$alkoxy and $-CO_2R^4$ where R^4 is as defined further above; and →O; it being understood that in the case where substituent R^{46} is present, that it results in said nitrogen atom and said moiety of partial Formula (4.0.0) is in quaternary form;

[R_{eglon} δ] is a member consisting of:

- an aryl and heterocyclyl-(substituted) amide, carbamate; or urea moiety of partial Formula (5.1.0):

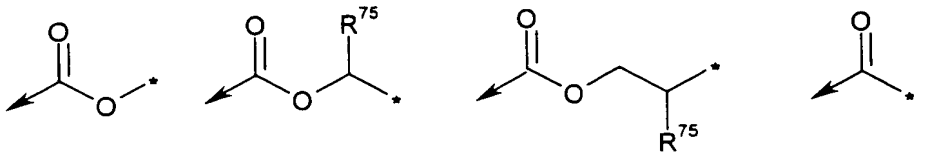


(5.1.0)

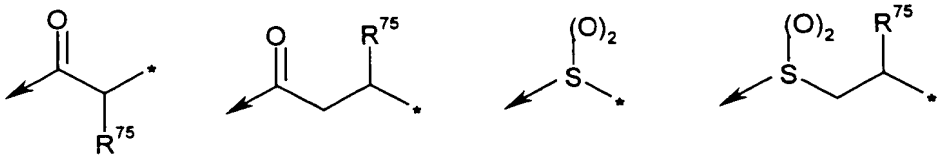
—wherein: the symbol " * " is as defined above;

—R^{73} is a member selected from the group consisting of hydrogen and $(C_1\text{-}C_2)$alkyl;

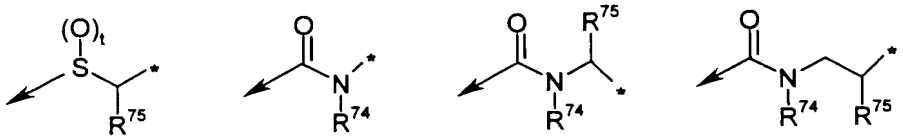
—W^5 is selected from the group consisting the moieties of partial Formulas (5.1.1) through (5.1.12):



(5.1.1) (5.1.2) (5.1.3) (5.1.4)



(5.1.5) (5.1.6) (5.1.7) (5.1.8)



(5.1.9) (5.1.10) (5.1.11) (5.1.12)

—wherein: the symbol: "→" indicates the point of attachment of the moiety W^5 represented by partial Formulas (5.1.1) through (5.1.12), inclusive, to the nitrogen atom in partial Formula (5.1.0), and the symbol: " * " indicates the point of attachment of the moiety W^5 to R^{82} as defined further below;

—R^{74} and R^{75} are each selected from the group consisting of hydrogen; $(C_1\text{-}C_2)$alkyl substituted by 0 or 1 substituent independently selected from OH; and $(C_1\text{-}C_2)$alkoxy; and

—R^{82} is a member selected from the group consisting of phenyl; cinnolinyl; furyl; thienyl; pyrrolyl; oxazolyl; isoxazolyl; thiazolyl; isothiazolyl; imidazolyl; imidazolinyl; pyrazolyl; pyrazolinyl; oxadiazolyl; thiadiazolyl; triazolyl; pyridyl; pyrazinyl; pyridazinyl; pyrimidinyl; parathiazinyl; indolyl; isoindolyl; indolinyl; benzo[*b*]furanyl; 2;3-dihydrobenzofuranyl; benzo[*b*]thiophenyl; 1H-indazolyl; benzimidazolyl; benzthiazolyl; quinolinyl; isoquinolinyl; phthalazinyl; quinazolinyl; quinoxalinyl; wherein:

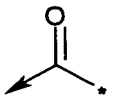
—the aryl or heterocyclyl moiety is substituted by 0 to 3 substituents R^{78}, where:

—R^{78} is a member selected from the group consisting of oxo; -Cl; -F; -OH; $-(C_1\text{-}C_2)$alkyl; $-(C_1\text{-}C_3)$alkoxy; $-CF_3$; -CN; $-C(=O)OR^{79}$; $-C(=O)NR^{79}R^{80}$; $-NR^{79}R^{80}$; $-NR^{79}C(=O)R^{80}$; $-NR^{79}C(=O)OR^{80}$; $-NR^{79}S(=O)_2R^{80}$; and $-S(=O)_2NR^{79}R^{80}$, where:

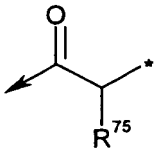
----R^{79} and R^{80} are each a member independently selected from the group consisting of hydrogen; and (C_1 .C_4)alkyl.

2. A compound as claimed in Claim 1, wherein the group of Formula (5.1.0) is selected from the group consisting of: carbamates, ureas and amides.

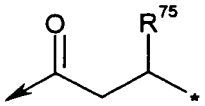
3. A compound ads claimes in Claim 2, wherein the group W^5 of Formula (5.1.0) is selected from the group consisting of partial Formulas (5.1.4) to (5.1.12):



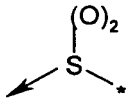
(5.1.4)



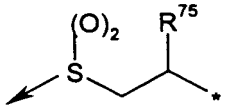
(5.1.5)



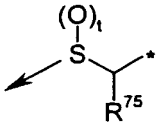
(5.1.6)



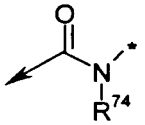
(5.1.7)



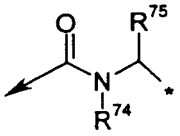
(5.1.8)



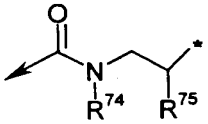
(5.1.9)



(5.1.10)

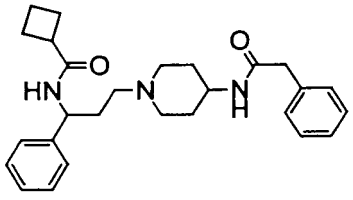


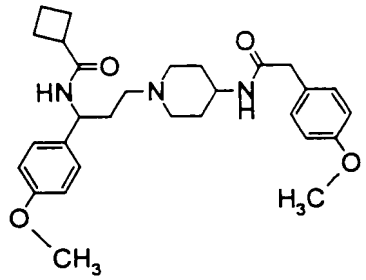
(5.1.11)

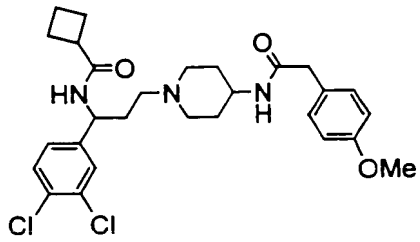


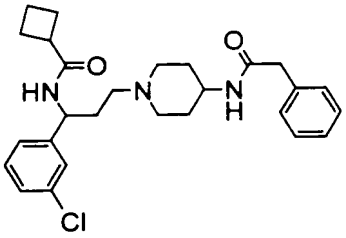
(5.1.12)

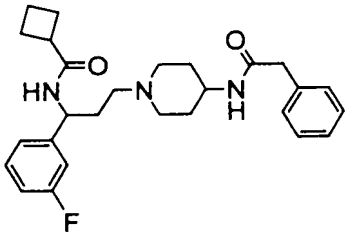
4. A compound which is selected from the group consisting of

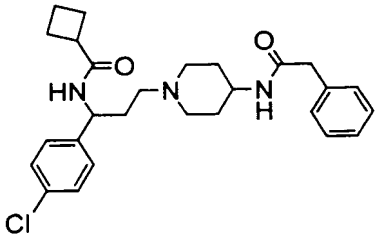


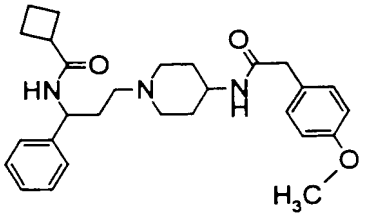












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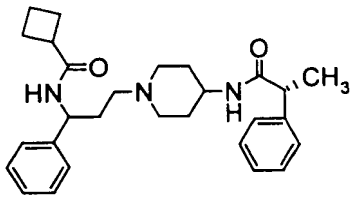

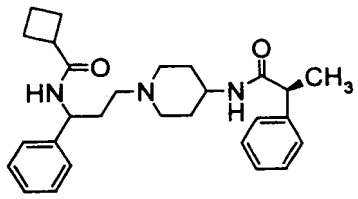

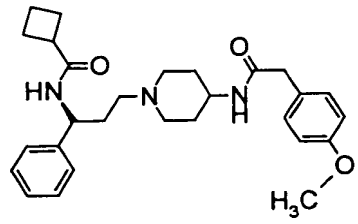

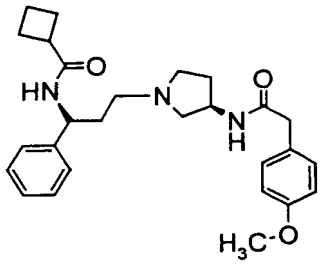

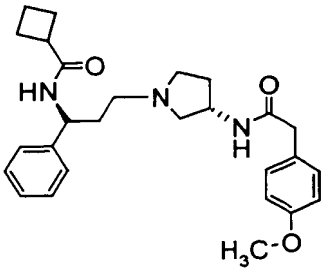

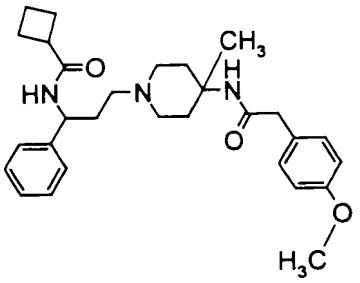

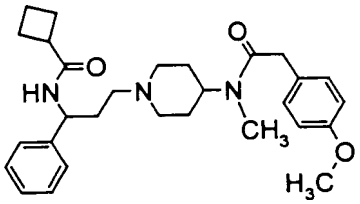

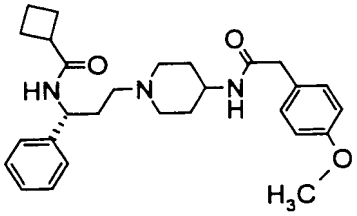

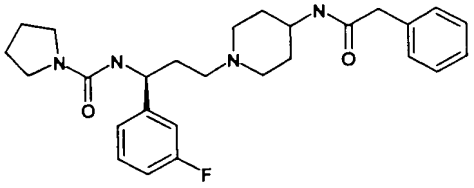

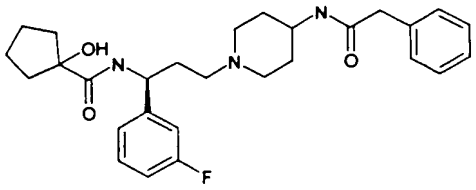

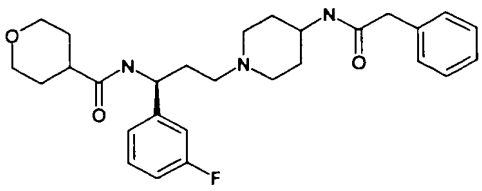

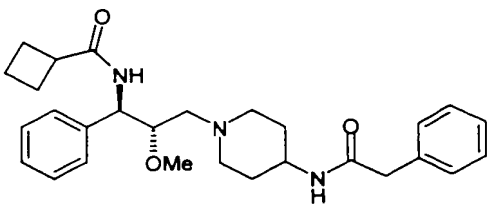

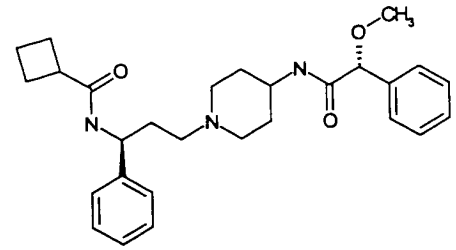

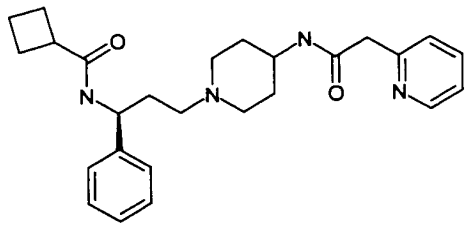

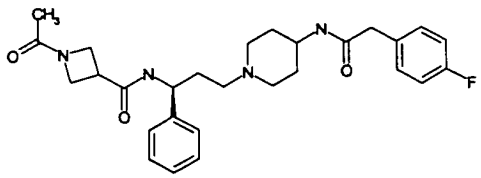

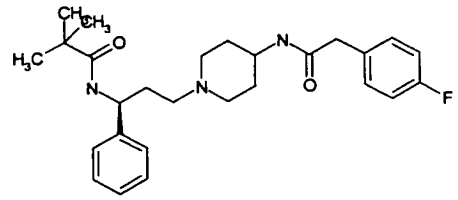

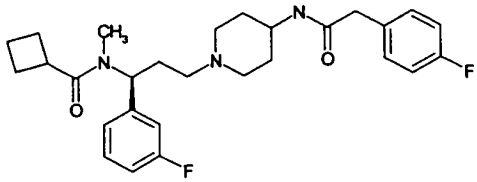

5

5. A compound which is selected from the group consisting of

N-(1-Phenyl-3-{4-[(2-phenylacetyl)amino]-1piperidinyl}propyl)cyclobutanecarboxamide

N-[3-(4-{[2-(4-Methoxyphenyl)acetyl]amino}-1-piperidinyl)-1-(4-methoxyphenylpropyl]cyclobutanecarboxamide

N-[3-(4-{[2-(4-Methoxyphenyl)acetyl]amino}-1-piperidinyl)-1-(3,4-dichlorophenylpropyl]cyclobutanecarboxamide

N-[3-(4-{[2-Phenylacetyl]amino}-1-piperidinyl)-1-(3-chlorophenylpropyl]cyclobutanecarboxamide

N-[3-(4-{[2-Phenylacetyl]amino}-1-piperidinyl)-1-(3-fluorophenylpropyl]cyclobutanecarboxamide

N-[3-(4-{[2-Phenylacetyl]amino}-1-piperidinyl)-1-(4-chlorophenylpropyl]cyclobutanecarboxamide

N-[3-(4-{[2-(4-Methoxyphenyl)acetyl]amino}-1-piperidinyl)-1-phenylpropyl]cyclobutanecarboxamide

N-[1-Phenyl-3-(4-{[(2*R*)-2-phenylpropanoyl]amino}-1-piperidinyl)propyl]cyclobutanecarboxamide

N-[1-Phenyl-3-(4-{[(2*S*)-2-phenylpropanoyl]amino}-1-piperidinyl)propyl]cyclobutanecarboxamide

N-[(1*S*)-3-(4-{[2-(4-methoxyphenyl)acetyl]amino}-1-piperidinyl)-1-phenylpropyl]cyclobutanecarboxamide

N-[(1*S*)-3-((3*R*)-3-{[2-(4-methoxyphenyl)acetyl]amino}pyrrolidinyl)-1-phenylpropyl]cyclobutanecarboxamide

N-[(1*S*)-3-((3*S*)-3-{[2-(4-methoxyphenyl)acetyl]amino}pyrrolidinyl)-1-phenylpropyl]cyclobutanecarboxamide

N-(3-{4-[[2-(4-methoxyphenyl)acetyl](methyl)amino]-1-piperidinyl}-1-phenylpropyl)cyclobutanecarboxamide

N-[3-(4-{[2-(4-methoxyphenyl)acetyl]amino}-4-methyl-1-piperidinyl)-1-phenylpropyl]cyclobutanecarboxamide

N-[(1*R*)-3-(4-{[2-(4-Methoxyphenyl)acetyl]amino}-1-piperidinyl)-1-phenylpropyl]cyclobutanecarboxamide

N-((1*S*)-1-(3-Fluorophenyl)-3-{4-[(2-phenylacetyl)amino]-1-piperidinyl}propyl)-1-pyrrolidinecarboxamide

N-((1*S*)-1-(3-Fluorophenyl)-3-{4-[(2-phenylacetyl)amino]-1-piperidinyl}propyl)-1-hydroxycyclopentanecarboxamide

N-((1*S*)-1-(3-Fluorophenyl)-3-{4-[(2-phenylacetyl)amino]-1-piperidinyl}propyl)tetrahydro-2*H*-pyran-4-carboxamide

N-((1*R*,2*S*)-2-Methoxy-1-phenyl-3-{4-[(2-phenylacetyl)amino]-1-piperidinyl}propyl)cyclobutanecarboxamide

N-[(1*S*)-3-(4-{[(2*R*)-2-methoxy-2-phenylethanoyl]amino}-1-piperidinyl)-1-phenylpropyl]cyclobutanecarboxamide

N-[(1*S*)-1-Phenyl-3-(4-{[2-(2-pyridinyl)acetyl]amino}-1-piperidinyl)propyl]cyclobutanecarboxamide

1-Acetyl-*N*-[(1*S*)-3-(4-{[2-(4-fluorophenyl)acetyl]amino}-1-piperidinyl)-1-phenylpropyl]-3-azetidinecarboxamide

N-[(1*S*)-3-(4-{[2-(4-Fluorophenyl)acetyl]amino}-1-piperidinyl)-1-phenylpropyl]-2,2-dimethylpropanamide

N-[(1*S*)-1-(3-Fluorophenyl)-3-(4-{[2-(4-fluorophenyl)acetyl]amino}-1-piperidinyl)propyl]-*N*-methylcyclobutanecarboxamide

6. A method of treating or preventing a disease or condition mediated by or associated with modulation of CCR5 chemokine receptor activity in a patient which is in need of such treatment or is a prospective beneficiary of such prevention, comprising administering to said patient an amount of a compound claimed in any preceding claim which is therapeutically effective to treat or prevent said disease or condition.

7. A pharmaceutical composition for treating or preventing a disease or condition mediated by or associated with modulation of CCR5 chemokine receptor activity comprising an amount of a compound claimed in any preceding claim which is therapeutically effective to treat or prevent said disease or condition, together with a pharmaceutically acceptable carrier therefor.

8. A method of treating or preventing infection by human immunodeficiency virus (HIV) in a patient which is in need of such treatment or is a prospective beneficiary of such prevention, including treatment or prevention of acquired immunodeficiency syndrome (AIDS) resulting therefrom, comprising administering to said patient an amount of a compound as claimed in any preceding claim which is therapeutically effective to treat or prevent said infection by HIV, including AIDS.

9. A method according to claim 8 further including coadministering to said patient in combination with a compound as claimed in any of claim 1 to 3, one or more additional therapeutic agents for treating or preventing HIV infection comprising one or more members selected from the group consisting of (1) inhibitors of HIV protease; and (2) inhibitors of HIV reverse transcriptase.

10. A method according to claim 9 wherein: (1) said inhibitors of HIV protease comprise one or more members selected from the group consisting of indinavir, ritonavir, saquinavir, nelfinavir, and amprenavir; and (2) said inhibitors of HIV reverse transcriptase comprise one or more members selected from the group consisting of (a) non-nucleoside reverse transcri8ptase inhibitors (NNRTIs) selected from nevirapine, delavirdine, and efavirenz; and (b) nucleoside/nucleotide inhibitors (NRTIs) selected from zidovudine, didanosine, zalcitabine, stavudine, lamivudine, abacavir, and adefovir dipivoxil.

11. A method according to claim 9 wherein said inhibitors of HIV protease and said inhibitors of HIV reverse transcriptase comprise one or more members selected from the

group consisting of indinavir; ritonavir' saquinavir; nelfinavir; amprenavir; nevirapine; elavirdine; efavirenz; zidovudine; didanosine; zalcitabine; stavudine; lamivudine; abacavir; adefovir dipivoxil; FTC; PMPA; fozivudine tidoxil; talviraline; S-1153; MKC-442; MSC-204; MSH-372; DMP450; PNU-140690; ABT-378; and KNI-764.

12. A method according to claim 9 wherein said method comprises preventing HIV infection and said patient being treated is aviremic and/or asymptomatic and is potentially or effectively infected with HIV, comprising administering to said patient a combination of therapeutic agents comprising a member selected from the group consisting of: (i) a compound as claimed in claim 1; (ii) one non-nucleoside reverse transcriptase inhibitor (NNRTI) in addition to a compound of (I); (iii) one nucleoside/nucleotide inhibitor (NRTI) in addition to a compound of (I); (iv) one NRTI in addition to the combination of (ii); and (v) a compound selected from inhibitors of HIV protease used in place of said NRTI in combinations (iii) and (iv).

13. A method according to claim 9 wherein said method comprises treating HIV infection and said patient being treated has detectable viremia or abnormally low CD4 counts, comprising administering to said patient a combination of therapeutic agents comprising (A) a member selected from the group consisting of a compound of Formula (I) as defined in claim 1; and a therapeutic agent comprising one protease inhibitor in combination with two NRTIs; or (B) the combination of therapeutic agents recited in (A) where either said protease inhibitor component, or one or both of said NRTIs is/are replaced by a compound of Formula (I) as defined in claim 1.

14. A method according to claim 9 wherein said method comprises treating HIV-infected individuals that have failed antiviral therapy comprising adminstering to said patient a combination of therapeutic agents comprising (A) a member selected from the group consisting of a compound as claimed in claim 1; or (B) a therapeutic agent comprising one protease inhibitor in combination with two NRTIs where either said protease inhibitor component, or one or both of said NRTIs is/are replaced by a compound of Formula (I) as defined in claim 1.

15. A method according to claim 10 further comprising coadministering with said compound of Formula (I) as defined in claim 1 one or more supplementary therapeutic agents which provide auxiliary treatment of diseases or conditions directly resulting from or indirectly accompanying infection by HIV, including AIDS resulting therefrom, wherein said supplementary therapeutic agent is one or more members selected from the group consisting of proliferation inhibitors; immunomodulators; interferon or interferon derivatives; fusion

inhibitors; integrase inhibitors; RnaseH inhibitors; and inhibitors of viral transcription and RNA replication.

16. A method according to claim 15 wherein said proliferation inhibitor is hydroxyurea; said immunomodulator is sargramostim; said fusion inhibitor is AMD3100, T-20, PRO-542, AD-349, or BB-10010; and said integrase inhibitor is AR177.

17. A pharmaceutical composition for treating or preventing infection by human immunodeficiency virus (HIV) in a patient which is in need of such treatment or is a prospective beneficiary of such prevention, including treatment or prevention of acquired immunodeficiency syndrome (AIDS) resulting therefrom, comprising an amount of a compound as claimed in claim 1 which is therapeutically effective to treat or prevent said infection by HIV or AIDS resulting therefrom, together with a pharmaceutically acceptable carrier therefor.

18. A pharmaceutical composition according to claim 17 further including in combination with a compound of Formula (I) as claimed in claim 1, one or more additional therapeutic agents for treating or preventing HIV infection comprising one or more members independently selected from the group consisting essentially of (1) inhibitors of HIV protease; and (2) inhibitors of HIV reverse transcriptase.

19. A pharmaceutical composition according to claim 18 wherein: (1) said inhibitors of HIV protease comprise one or more members independently selected from the group consisting of indinavir, ritonavir, saquinavir, nelfinavir, and amprenavir; and (2) said inhibitors of HIV reverse transcriptase comprise one or more members selected from the group consisting of (a) non-nucleoside reverse transcriptase inhibitors selected from nevirapine, delavirdine, and efavirenz; and (b) nucleoside/nucleotide inhibitors selected from zidovudine, didanosine, zalcitabine, stavudine, lamivudine, abacavir, and adefovir dipivoxil.

20. A pharmaceutical composition according to claim 18 wherein said inhibitors of HIV protease and said inhibitors of HIV reverse transcriptase comprise one or more members selected from the group consisting of indinavir; ritonavir; saquinavir; nelfinavir; amprenavir; nevirapine; delavirdine; efavirenz; zidovudine; didanosine; zalcitabine, stavudine; lamivudine; abacavir; adefovir dipivoxil; FTC; PMPA; fozivudine todoxil; talviraline; S-1153; MKC-442; MSC-204; MSH-372; DMP450; PNU-140690; ABT-378; and KNI-764.

21 A pharmaceutical composition according to claim 17 further comprising coadministering with said compound of Formula (1) as defined in claim 1 one or more

supplementary therapeutic agents which provide auxiliary treatment of diseases or conditions directly resulting from or indirectly accompanying infection by HIV, including AIDS resulting therefrom, wherein said supplementary therapeutic agent is one or more members selected from the group consisting of proliferation inhibitors; immunomodulators; interferon or interferon derivatives; fusion inhibitors; integrase inhibitors; RNaseH inhibitors; and inhibitors of viral transcription and RNA replication.

22. A pharmaceutical composition according to claim 21 wherein said proliferation inhibitor is hydroxyurea; said immunomodulator is sargramostim; said fusion inhibitor is AMD3100, T-20, PRO-542, AD-349 or BG-10010; and said integrase inhibitor is AR177.

23. A method of evaluating a putative HIV retrovirus mutant for resistance to anti-HIV therapeutic agents, comprising isolating said putative mutant virus from an *in vitro* culture thereof; an *in vitro* animal infection model thereof; or from patient samples where said patient is undergoing optimal or sub-optimal treatment comprising administration of a compound as defined in claim 1, alone or together in any combination thereof with one or more therapeutic agents for treating or preventing HIV infection.

24. A mutant HIV virus or component part thereof, prepared in accordance with the procedures of claim 23.

25. A mutant HIV virus or component thereof according to claim 23 wherein said component is the complete envelope protein thereof, or infections fragment thereof.

26. A method of discovering the presence of, and/or confirming the activity of a chemokine modulator having activity against a mutant HIV virus, comprising using as a probe for effecting said discovery and/or confirmation a mutant HIV virus or component thereof according to claim 23.

27. A diagnostic agent for use in choosing a therapeutic regimen and/or predicting the outcome for a patient being treated for infection by a mutant HIV virus, wherein said diagnostic agent comprises a mutant HIV virus or component thereof according to claim 21.

28. A pharmaceutical composition for treating or preventing a respiratory disease or condition comprising an amount of a compound claimed in any of claims 1 to 5 which is effective to treat said disease or condition, together with a pharmaceutically effective carrier therefor.

29. A compound as claimed in claims 1 to 5 in purified form.

30. A pharmaceutical composition comprising a compound as claimed in any of claims 1 to 5 and one or more inert excipients.

ABSTRACT

Compounds of Formula 1

$$[R_{egion}\ \alpha] - [R_{egion}\ \beta] - [R_{egion}\ \gamma] - [R_{egion}\ \delta] \qquad (I)$$

which are useful as modulators of chemokine activity. The invention also provides pharmaceutical formulations and methods of treatment using these compounds.